UNIVERSITI PUTRA MALAYSIA

PHYLOGENETIC RELATIONSHIPS AND POPULATION STRUCTURE OF Portunus pelagicus IN THE COASTAL WATERS OF MALAYSIA INFERRED FROM MITOCHONDRIAL DNA AND MICROSATELLITES

CHAI CHUAN JIAN

FP 2017 28

PHYLOGENETIC RELATIONSHIPS AND POPULATION STRUCTURE OF *Portunus pelagicus* IN THE COASTAL WATERS OF MALAYSIA INFERRED FROM MITOCHONDRIAL DNA AND MICROSATELLITES

By

CHAI CHUAN JIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

PHYLOGENETIC RELATIONSHIPS AND POPULATION STRUCTURE OF *Portunus pelagicus* IN THE COASTAL WATERS OF MALAYSIA INFERRED FROM MITOCHONDRIAL DNA AND MICROSATELLITES

By

CHAI CHUAN JIAN

July 2017

Chairman: Yuzine Bin Esa, PhD
Faculty: Agriculture

The abundance of blue swimmer crabs, *Portunus pelagicus* throughout the Indo-West Pacific region makes it a valuable target species for fishery industry. The increasing demands of *P. pelagicus* have led to a growing interest on the broodstock selection of this particular crab species. This study examined the phylogenetic relationships, demographic history and population structure of *P. pelagicus* through sequence analyses of mitochondrial cytochrome c oxidase I (COI) gene (600 bp) as well as fragment analyses of four pairs of microsatellite loci. A total of 109 crab samples were collected from five different populations throughout the coastal areas of Malaysia. These include Perak (Pantai Remis and Kuala Sepetang), Johor (Pendas), Negeri Sembilan (Port Dickson), Terengganu (Besut) and Sarawak (Bako).

In total, 15 haplotypes were obtained with 13 unique haplotypes and two shared haplotypes. The phylogenetic analyses via the Neighbour Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) methods supported the monophyletic status between *P. pelagicus* and *P. sanguinolentus* with high bootstrap value (100%). All three phylogenetic trees revealed similar tree topologies with differences in the bootstrap values. The clustering of all *P. pelagicus* samples into a single clade suggested that this species possibly belonged to a single species.

The genetic identity of *P. pelagicus* was further elucidated with low genetic distances among the haplotypes (0.2-1.7%) via mitochondrial analyses. Limited variations were found among the populations of *P. pelagicus* with high haplotype diversity and low nucleotide diversity detected within each population. No hybrid individual was discovered based on the genetic analyses of *P. pelagicus* from sympatric sampling locations. Low F_{ST} values obtained among these populations also clarified that these crabs were compatible for breeding programs.

One interesting finding of this study was the sharing of haplotypes between samples from Sarawak and Peninsular Malaysia. This situation proclaimed that the rise of sea levels during the last Pleistocene did not result in isolation of *P. pelagicus* populations from Sarawak, thus, allowed the migration and gene flow between both continents. Another remarkable result was the significant levels of genetic differentiation deduced through comparisons of *P. pelagicus* from Terengganu and the other four populations. The occurrence might be explained with overexploitation in the fishery industries across the coastal areas of Terengganu and the limited number of crab samples (n=25) analysed.

Microsatellite analyses on the other hand indicated low levels of genetic differentiation among the *P. pelagicus* populations. The average observed heterozygosity (H_O=0.48) obtained was lower than the standard heterozygosity found in most marine populations (H_O=0.79). The high F_{IS} values (mean F_{IS}=0.4756) and low F_{ST} values (mean F_{ST}=0.0413) also suggested the existence of inbreeding among different populations of *P. pelagicus*. When compared with the outcomes from microsatellite analyses, mitochondrial dataset tended to show higher sensibility in detection of genetic variations.

In conclusion, the present study was able to provide knowledge on the phylogenetic relationships, demographic history and population structure of *P. pelagicus* in coastal areas of Malaysia. Nevertheless, in order to better unravel the degrees of genetic differentiation within and among the populations, larger scale studies with higher population size and types of genetic markers used need to be implemented.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Sarjana Sains

HUBUNGAN FILOGENETIK DAN STRUKTUR POPULASI *Portunus pelagicus* DI KAWASAN PERAIRAN PANTAI MALAYSIA DISIMPULKAN DARIPADA DNA MITOKONDRIA DAN MIKROSATELIT

Oleh

CHAI CHUAN JIAN

Julai 2017

Pengerusi: Yuzine Bin Esa, PhD
Fakulti: Pertanian

Bilangan *Portunus pelagicus* atau lebih dikenali sebagai ketam biru yang banyak di seluruh rantau Indo Pasifik Barat menjadikan ianya spesies sasaran yang bernilai tinggi dalam industri perikanan. Permintaan *P. pelagicus* yang semakin meningkat kini menarik perhatian ramai dalam pemilihan stok induk bagi spesies ketam ini. Kajian ini menguji hubungan filogenentik, sejarah demografi dan struktur populasi *P. pelagicus* melalui analisis jujukan menggunakan gene (600 bp) mitokondria sitokrom c oksides I (COI) dan analisis fragmen menggunakan empat pasangan penanda mikrosatelit. Sebanyak 109 sampel ketam telah dikumpul dari lima populasi berbeza di sekitar kawasan persisiran pantai Malaysia. Antaranya termasuk Perak (Pantai Remis and Kuala Sepetang), Johor (Pendas), Negeri Sembilan (Port Dickson), Terengganu (Besut) and Sarawak (Bako).

Secara keseluruhan, 15 haplotaip telah diperoleh dengan 13 haplotaip unik dan dua haplotaip kongsian. Analisis filogenetik melalui kaedah Neighbour Joining (NJ), Maximum Parsimony (MP) dan Maximum Likelihood (ML) menyokong status monofiletik antara *P. pelagicus* dan *P. sanguinolentus* dengan nilai bootstrap yang tinggi (100%). Ketiga-tiga pokok filogenetik mendedahkan topologi pokok yang serupa dengan perbezaan pada nilai bootstrap. Pengelompokan kesemua sampel *P. pelagicus* dalam satu cabang mencadangkan bahawa spesies ini berkemungkinan besar merupakan satu spesies tunggal.

Identiti genetik *P. pelagicus* selanjutnya dijelaskan dengan jarak genetik yang rendah di kalangan haplotaip (0.2-1.7%) melalui analisis mitokondria. Variasi yang terhad didapati di kalangan populasi *P. pelagicus* dengan kepelbagaian haplotaip yang tinggi dan kepelbagaian nukleotida yang rendah dikesan dalam setiap populasi. Tiada individu hibrid didedahkan berdasarkan analisis genetik *P. pelagicus* dari lokasi persampelan simpatrik. Nilai F_{ST} rendah yang diperolehi di kalangan populasi turut mengesahkan bahawa ketam-ketam ini sesuai untuk program pembiakan.

Satu penemuan yang menarik dalam kajian ini adalah perkongsian haplotaip antara sampel dari Sarawak dan Semenanjung Malaysia. Situasi ini membuktikan bahawa kenaikan paras laut pada Pleistosen lepas tidak mengakibatkan perasingan populasi *P. pelagicus* dari Sarawak, justeru, membenarkan penghijrahan dan aliran gen antara kedua-dua benua. Selain itu, tahap perbezaan genetik yang ketara dapat disimpulkan daripada perbandingan antara *P. pelagicus* dari Terengganu dan empat populasi yang lain. Fenomena ini dapat dijelaskan dengan eksploitasi yang tinggi dalam industri perikanan di kawasan persisiran pantai Terengganu dan jumlah sampel ketam (n=25) yang dianalisa terhad.

Analisis microsatelit pula menunjukkan tahap perbezaan genetik yang rendah di kalangan populasi *P. pelagicus*. Nilai purata heterozigositi (H_O=0.48) yang diperoleh adalah lebih rendah berbanding dengan nilai purata heterozigositi piawai (H_O=0.79) yang didapati dalam kebanyakan populasi marin. Nilai F_{IS} (purata F_{IS}=0.4756) yang tinggi dan nilai F_{ST} (purata F_{ST}=0.0413) yang rendah juga mencadangkan kewujudan pembiakan dalaman antara populasi *P. pelagicus* yang berlainan. Ketika dibandingkan dengan keputusan analisis mikrosatelit, dataset mitokondria menunjukkan kepekaan yang lebih tinggi dalam pengesanan variasi genetik.

Kesimpulannya, kajian ini berupaya memberikan pengetahuan tentang hubungan filogenetik, sejarah demografi dan struktur populasi *P. pelagicus* di kawasan persisiran pantai Malaysia. Walaubagaimanapun, untuk lebih memahami tahap perbezaan genetik dalam dan di kalangan populasi, kajian dengan skala yang lebih besar dari segi saiz populasi dan jenis penanda genetik yang digunakan perlu dijalankan.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my truthful appreciation to my research supervisor, Assoc. Prof. Dr. Yuzine Bin Esa and co-supervisor, Dr. Muhammad Fadhil Syukri Ismail for their guidance and encouragement throughout this research study. They have contributed much towards my understanding and thoughts in regard to this particular research title. I am also indebted to my beloved family members especially my parents for their spiritual and financial supports throughout this research. Without their motivation and advices, the study would not have been a success. Once again, thank you.

In addition, I would like to extend my gratitude towards staff members of the Aquaculture Department UPM. In particular, thanks to Mr. Mohamad Zawawi Idris for his assistance during my laboratory sessions. Not to be forgotten, all the staffs from Fisheries Department of Malaysia who deserve special thanks for their collaboration throughout my fieldwork. I would also like to take this opportunity to thank my seniors and friends, namely Izzati Azmir, Mohd. Azim, Nurnadia Marshita, Puvaneswari Puvanasundram, Syakina Rahim, Intan Sukimin and Nona Tarmizi. They always give a hand without hesitation whenever necessary.

Last but not least, I wish to express my appreciation to everyone involved in this research study either directly or indirectly. Thank you for your cooperation. Thank you.

CHAI CHUAN JIAN
09 September 2016

I certify that a Thesis Examination Committee has met on 17 July 2017 to conduct the final examination of Chai Chuan Jian on his thesis entitled "Phylogenetic Relationships and Population Structure of *Portunus pelagicus* in the Coastal Waters of Malaysia Inferred from Mitochondrial DNA and Microsatellites" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

S. M. Nurul Amin, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mohd Zamri bin Saad, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Siti Azizah Mohd. Nor, PhD
Associate Professor
Universiti Sains Malaysia
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 September 2017

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Yuzine Bin Esa, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Muhammad Fadhil Syukri Ismail, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- This thesis is my original work;
- Quotations, illustrations and citations have been duly referenced;
- This thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- Intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- Written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- There is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: ________________________ Date: ___________________

Name and Matric No.: Chai Chuan Jian / GS41984

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: ______________________________
Name of
Chairman of
Supervisory
Committee: Yuzine Bin Esa

Signature: ______________________________
Name of
Member of
Supervisory
Committee: Muhammad Fadhil Syukri Ismail

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

^oC	Degree Celsius
m	Meter
cm	Centimeter
mm	Millimeter
mg	Milligram
mM	Millimolar
ml	Milliliter
µl	Microliter
bp	Base pair
kb	Kilobase
rpm	Revolutions per minute
dNTP	Deoxynucleotide triphosphate
UV	Ultra violet
V	Volt
DNA	Deoxyribonucleic acid
$MgCl_2$	Magnesium chloride
TBE	Tris/Borate/EDTA
LE	Low electroendosmosis
HR	High resolution
ATL	Animal tissue lysis
AL	Animal lysis
AW	Animal wash
AE	Animal elution
NCBI	National Center for Biotechnology Information
CI	Consistency index
RI	Retention index
HI	Homoplasy index
ML	Maximum Likelihood
MP	Maximum Parsimony
NJ	Neighbour Joining

CHAPTER 1

INTRODUCTION

1.1 Research Background

The *Origin of Species* published by Charles Darwin in 1849 marked the emergence of phylogeny, a discipline of classifying all living organisms (Sleator, 2011). Modern phylogenetic study is the study on the evolutionary relationships among groups of organisms. In order to verify the phylogenetic relationships among organisms, both morphological and molecular data can be used. Nevertheless, traditional morphology-based phylogeny approaches have significant limitation in reconstructing evolutionary event that required enormous character changes (Sleator, 2010). The advent development of molecular study has effectively eliminated this limitation. Molecular data are now easily obtained from gene sequences. Throughout the years, many phylogenetic studies based on molecular data had been carried out in different groups of Portunidae (Rosly et al., 2013; Naim et al., 2012; Mantelatto et al., 2007; Chu et al., 1999).

In phylogenetic study, genetic diversity can be defined as the study on varieties of alleles and genotypes present in populations which are then reflected in the morphological, physiological and behavioural differences between individuals or populations (Reed and Frankham, 2003). In the early 20th century, the World Conservation Union (IUCN) has recognised genetic variations as one of the three forms of biodiversity for conservation (McNeely et al., 1990). As wild species and domestic breeds are diminishing at an alarming rate, human intervention is required to assure their survivals (Frankham et al., 2002).

Portunidae, a family of crabs more commonly known as the swimming crabs, has encountered intense fishing pressure since the 1950s due to its high economic value (Yu et al., 2004). Therefore, information on the population structure of the portunid crabs is essential for development of effective strategies for fishery management (Xu and Liu, 2011). In spite of the commercial importance, there are a few portunid crabs' genera that have been systematically revised. *Charybdis* is one of the largest genera of portunid crabs with 64 species recorded in the Indo-West Pacific region including the newly described species, *Charybdis goaensis* (Padate et al., 2010; Ng et al., 2008). Besides, there are at least 11 described species of the genus *Callinectes* that inhabit temperate and tropical waters of the Atlantic and Pacific oceans (Place et al., 2005). Mud crab of the genus *Scylla* consists of four distinct species (Klinbunga et al., 2010).

The genus *Portunus* on the other hand assigned over 90 extant species worldwide (Grave et al., 2009). In the western Atlantic, 14 species of *Portunus* crabs have been presently recognized but 12 of them are classified as valid species based on molecular analyses (Sanvicente-Anorve et al., 2008; Mantelatto et al., 2007). The abundance of blue swimmer crab, *Portunus pelagicus* across the Indo-West Pacific region makes it a valuable target species for fishery industry (Lai et al., 2010). In Malaysia, the genetic status of *P. pelagicus* is poorly known. Recent studies conducted on *P. pelagicus* in Malaysia mostly related to the nutritional ecology of this particular species (Ikhwanuddin et al., 2014). Previous genetic analyses in line with morphometric and morphological studies proclaimed that *P. pelagicus* is a four-member species complex (Sienes et al., 2014; Lai et al., 2010). Hence, further studies on *P. pelagicus* are crucial to gain precise genetic information for future planning.

1.2 Research Justification

There have been limited or no publication concerning the genetic diversity of *Portunus pelagicus* in coastal areas of Malaysia. Based on Lai et al. (2010) systematic revision, *P. pelagicus* is a species complex separated into four discrete species. Sienes et al. (2014) revealed the existence of a cryptic species within *P. pelagicus* in Philippines waters. This raised the notion that *P. pelagicus* in Malaysian waters might experience genetic variations likewise. Recognition of reproductively isolated and genetically differentiated populations within a species is of importance for broodstock selection and breeding programs (Conover et al., 2006; Carvalho and Hauser, 1994). Thus, the purpose of this current research is to examine the genetic diversity and intraspecific population differentiation of *P. pelagicus* in Malaysian waters via mitochondrial DNA and microsatellites analyses for which no datum is available at present. The basic information obtained can be applied to the construction of a genetic-based stock enhancement program and to avoid including inbred founder populations in breeding programs.

1.3 Research Aim

The aim of this study is to clarify the genetic diversity of *Portunus pelagicus* in coastal areas of Malaysia. This aim is achieved by elucidating the phylogenetic relationships and population structure among different populations of *P. pelagicus* through mitochondrial DNA as well as microsatellite primers.

1.4 Research Objectives

The objectives of this research are as follows:

a) To generate the phylogenetic relationships of *Portunus pelagicus* in coastal areas of Malaysia using mitochondrial DNA.
b) To determine the population structure of *P. pelagicus* in coastal areas of Malaysia using microsatellites.

1.5 Research Questions

The research questions for this study are as follows:

1. Could the mitochondrial cytochrome c oxidase subunit I (COI) gene resolve the phylogenetic relationships among *Portunus pelagicus* in Malaysia?
2. Could the microsatellite primers be able to unravel the population structure of *P. pelagicus* in Malaysia?

1.6 Research Scope

The blue swimmer crab, *Portunus pelagicus,* was selected as the target species for this research. Another species from the genus *Portunus*, namely *P. sanguinolentus* was involved in this research for comparison purposes, both morphologically and genetically. On the contrary, genus *Charybdis* was chosen as an outgroup. Samples were collected from five coastal areas of Malaysia which include Perak (Pantai Remis and Kuala Sepetang), Johor (Pendas), Negeri Sembilan (Port Dickson), Terengganu (Besut) and Sarawak (Bako).

REFERENCES

Arshad, A., Efrizal, Kamarudin, M.S. and Saad, C.R. 2006. Study on Fecundity, Embryology and Larval Development of Blue Swimming Crab *Portunus pelagicus* (Linnaeus, 1758) Under Laboratory Conditions. *Res. J. Fish. Hydrobiol.*, 1(1): 35-44.

Avise, J.C. 1994. *Molecular Markers, Natural History and Evolution*. New York: Chapman and Hall.

Avise, J.C. 2000. *Phylogeography: The History and Formation of Species*. Cambridge: Harvard University Press.

Balloux, F. and Goudet, J. 2002. Statistical Properties of Population Differentiation Estimators under Stepwise Mutation in a Finite Island Model. *Molecular Ecology*, 11: 771-783.

Balloux, F. and Lugon-Moulin, N. 2002. The Estimation of Population Differentiation with Microsatellite Markers. *Molecular Ecology*, 11: 155-165.

Bartlett, S.E. and Davidson, W.S. 1991. Identification of *Thunnus* Tuna Species by the Polymerase Chain Reaction and Direct Sequence Analysis of Their Mitochondrial Cytochrome b Genes. *Canadian Journal of Fisheries and Aquatic Science*, 48: 309-317.

Biodiversity Heritage Library. *Original description of Cancer pelagicus*. Retrieved from http://www.biodiversitylibrary.org/648/mode.

Bohonak, A.J. and Jenkins, D.G. 2003. Ecological and Evolutionary Significance of Dispersal by Freshwater Invertebrates. *Ecology Letters*, 6: 783-796.

Borradaile, L.A. 1902. Marine crustaceans. III. The Xanthidae and Other Crabs. *In*: Gardiner, J.S. (Ed.). *The Fauna and Geography of the Maldive and Laccadive Archipelagoes. Volume 1*. Cambridge: Cambridge University Press, pp. 199.

Brook, B.W., Tonkyn, D.W., O'Grady, J.J. and Frankham, R. 2002. Contribution of Inbreeding to Extinction Risk in Threatened Species. *Conservation Ecology*, 6(1): 16.

Brown, L.D., Gunter, P.T., Waters, M.J. and Epifanio, M.J. 2000. Evaluating Genetic Diversity Associated with Propagation Assisted Restoration of American Shad. *Conserv. Biol.*, 14: 294-303.

Bruford, M.W. and Wayne, R.K. 1993. Microsatellites and Their Application to Population Genetic Studies. *Genetics and Development*, 3: 939-943.

Bryars, S.R. and Adams, M. 1999. An Allozyme Study of the Blue Swimmer Crab, *Portunus pelagicus* (Crustacea: Portunidae), in Australia: Stock Delineation in Southern Australia and Evidence for a Cryptic Species in Northern Waters. *Marine and Freshwater Research*, 50: 15-26.

Buhay, J.E. and Crandall, K.A. 2005. Subterranean Phylogeography of Freshwater Crayfishes Shows Extensive Gene Flow and Surprisingly Large Population Sizes. *Molecular Ecology*, 14: 4259-4273.

Callen, D.F., Thompson, A.D., Shen, Y., Philips, H.A., Richards, R.I., Mulley, J.C. and Sutherland, G.R. 1993. Incidence and Origin of "Null" Alleles in the (AC)n Microsatellite Markers. *Am. J. Hum. Genet.*, 52: 922-927.

Campbell, G.R. and Fielder, D.R. 1986. Size at Sexual Maturity and Occurrence of Ovigerous Females in Three Species of Commercially Exploited Portunid Crabs in SE Queensland. *Proceedings of the Royal Society of Queensland*, 97: 79-87.

Carvalho, G.R. and Hauser, L. 1994. Molecular Genetics and the Stock Concept in Fisheries. *Reviews in Fish Biology and Fisheries*, 4: 326-350.

Chakraborty, R., Kimmel, M., Stivers, D.N., Davison, L.J. and Deka, R. 1997. Relative Mutation Rates at Dinucleotide, Trinucleotide and Tetranucleotide Microsatellite. *Proc. Natl. Acad. Sci. USA*, 94: 1041-1046.

Chan, S.C., Siraj, S.S., Tan, S.G. and Yusoff, K. 2005. Rapid and Non-radioactive Detection Method of Microsatellites in *Mystus nemurus:* A Refined Technique. *Pertanika J. Trap. Agric. Sci.*, 28(1): 73-77.

Chande, A.I. and Mgaya, Y.D. 2003. The Fishery of *Portunus pelagicus* and Species Diversity of Portunid Crabs along the Coast of Dar es Salaam, Tanzania*. Western Indian Ocean J. Mar. Sci.*, 2(1): 75-84.

Chaplin, J., Yap, E.S., Sezmis, E. and Potter, I.C. 2001. *Genetic (Microsatellite) Determination of the Stock Structure of the Blue Swimmer Crab in Australia*. FRDC Project 98/118.

Chatterji, S. and Pachter, L. 2006. Reference Based Annotation with GeneMapper. *Genome Biology*, 7(4): R29.

Chu, K.H., Tong, J.G. and Chan T.Y. 1999. Mitochondrial Cytochrome Oxidase I Sequence Divergence in Some Chinese Species of *Charybdis* (Crustacea: Decapoda: Portunidae). *Biochemical Systematics and Ecology*, 27: 461-468.

Clare, E.L., Lim, B.K., Engstrom, M.D., Eger, J.L. and Hebert, P.D.N. 2007. DNA Barcoding of Neotropical Bats: Species Identification and Discovery within Guyana. *Molecular Ecology Notes*, 7: 184-190.

Conover, D.O., Clarke, L.M., Munch, S.B. and Wagner, G.N. 2006. Spatial and Temporal Scales of Adaptive Divergence in Marine Fishes and the Implications for Conservation. *Journal of Fish Biology*, 69: 21-47.

Costa, F.O., deWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M. and Hebert, P.D.N. 2007. Biological Identifications through DNA Barcodes: The Case of Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences*, 64: 272-295.

Davie, P.J.F. 2002. Crustacea: Malacostraca: Eucarida (Part 2): Decapoda-Anomura, Brachyura. *In*: Wells, A. and Houston, W.W.K. (Eds.). *Zoological Catalogue of Australia. Volume 19.3B*. Melbourne: CSIRO Publications, pp. 641.

de Lestang, S., Platell, M.E. and Potter, I.C. 2000. Dietary Composition of the Blue Swimming Crab *Portunus pelagicus* L. Does It Vary with Body Size and Shell State and Between Estuaries? *Journal of Experimental Marine Biology and Ecology*, 246(1): 241-257.

Department of Fisheries. 2009. *Fisheries Statistics of Malaysia in 2009*. Fisheries Statistics of Malaysia, Fishery Economic Division, Malaysia, pp. 329.

Department of Fisheries. 2011. *Fact Sheet No. 2: Blue Swimmer Crab*. Retrieved from http://www.fish.wa.gov.au/bsc.pdf.

Dewoody, J.A. and Avise, J.C. 2000. Microsatellite Variation in Marine, Freshwater and Anadromous Fishes Compared with Other Animals. *Journal of Fish Biology*, 56: 461-473.

Di Rienzo, A., Peterson, A.C., Garza, J.C., Valdes, A.M., Slatkin, M. and Freimer, N.B. 1994. Mutational Processes of Simple-sequence Repeat Loci in Human Populations. *Proceedings of the National Academy of Sciences of the United States of America*, 91(8): 3166-3170.

Dong, Z.G., Li, X.Y., Yan, B.L. and Sun X.W. 2012. Eighteen Novel Polymorphic Microsatellites for the Swimming Crab (*Portunus trituberculatus*). *Conservation Genet. Resour.*, 4: 825-828.

Edgar, G.J. 1990. Predator-prey Interactions in Seagrass Beds. II. Distribution and Diet of the Blue Manna Crab *Portunus pelagicus* Linneaus at Cliff Head, Western Australia. *Journal of Experimental Marine Biology and Ecology*, 139: 23-32.

Edwards, C.A. and Skibinski, D.O.E. 1987. Genetic Variation of Mitochondrial DNA in Mussel (*Mytilus edulis* and *M. galloprovincialis*) Populations from South West England and South Wales. *Mar. Biol.*, 94: 547-556.

Esa, Y.B. and Rahim, K.A.A. 2013. Genetic Structure and Preliminary Findings of Cryptic Diversity of the Malaysian Mahseer (*Tor tambroides* Valenciennes: Cyprinidae) Inferred from Mitochondrial DNA and Microsatellite Analyses. *BioMed Research International*, 2013: 1-14.

Esa, Y.B., Siraj, S.S., Daud, S.K., Ryan, J.J.R., Rahim, K.A.A. and Tan, S.G. 2008. Mitochondrial DNA Diversity of *Tor tambroides* Valenciennes (Cyprinidae) from Five Natural Populations in Malaysia. *Zoological Studies*, 47(3): 360-367.

Esa, Y.B., Siraj, S.S., Rahim, K.A.A., Daud, S.K., Ho, G.H., Tan, S.G. and Syukri, M.F. 2011. Genetic Characterization of Two Mahseer Species (*Tor douronensis* and *Tor tambroides*) Using Microsatellite Markers from Other Cyprinids. *Sains Malaysiana*, 40(10): 1087-1095.

Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the Number of Clusters of Individuals Using the Software Structure: A Stimulation Study. *Molecular Ecology*, 14: 2611-2620.

Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin ver. 3.0: An Integrated Software Package for Population Genetics Data Analysis. *Evolutionary Bioinformatics*, 1: 47-50.

FAO. *Species Fact Sheets Portunus pelagicus (Linnaeus, 1758)*. Retrieved from http://www.fao.org/fishery/species/2629/en.

FAO, 2007. *Yearbooks of Fisheries Statistics: Capture Production*. Rome: Stylus Pub. Llc., pp. 539.

Felsenstein, J. 1985. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution*, 7: 1193-1204.

Feral, J.P. 2002. How Useful are the Genetic Markers in Attempts to Understand and Manage Marine Biodiversity? *J. Exp. Mar. Biol. Ecol.*, 268: 121-145.

Fielder, D.R. and Eales, A.J. 1972. Observations on Courtship, Mating and Sexual Maturity in *Portunus pelagicus* (L., 1766) (Crustacea, Portunidae). *Journal of Natural History*, 6: 273-277.

Foka, M.C., Kondylatos, G. and Economidis, P.S. 2004. Occurrence of the Lessepsian Species *Portunus pelagicus* (Crustacea) and *Apogon* *pharaonis* (Pisces) in the Marine Area of Rhodes Island. *Mediterranean Marine Science*, 5(1): 83-89.

France, S.C. and Hoover, L.L. 2002. DNA Sequences of the Mitochondrial COI Gene have Low Levels of Divergence among Deep-sea Octocorals (Cnidaria: Anthozoa). *Hydrobiology*, 471: 149-155.

Frankham, R., Briscoe, D.A. and Ballou, J.D. 2002. *Introduction to Conservation Genetics.* New York: Cambridge University Press.

Fratini, S. and Vannini, M. 2002. Genetic Differentiation in the Mud Crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. *Journal of Experimental Marine Biology and Ecology*, 272: 103-116.

Fu, Y.X. 1997. Statistical Tests of Neutrality of Mutations against Population Growth, Hitch-hiking and Background Selection. *Genetics*, 147: 915-925.

Fujaya, Y., Asphama, A.I., Hidayani, A.A., Parenrengi, A. and Tenriulo A. 2016. High Genetic Variation of *Portunus pelagicus* from Makassar Straits Revealed by RAPD Markers and Mitochondrial 16S rRNA Sequences. *African Journal of Biotechnology*, 15(7): 180-190.

Fujaya, Y., Trijuno, D.D., Aslamyah, S. and Alam, N. 2016. Domestication and Selective Breeding for Producing Fast Growing and High Meat Quality of Blue Swimming Crab (*Portunus pelagicus*). *AACL Bioflux*, 9(3): 670-679.

Gao, B.Q., Liu, P., Li, J. and Dai, F.Y. 2007. Isozyme Polymorphism in *Portunus trituberculatus* from Wild Population. *J. Fish. China*, 31: 1-6.

Gao, B.Q., Liu, P., Li, J., Wang, Q.Y. and Li, X.P. 2014. Genetic Diversity of Different Populations and Improved Growth in the F1 Hybrids in the Swimming Crab (*Portunus trituberculatus*). *Genet. Mol. Res.*, 13(4): 10454-10463.

Gardner, J.P.A. 1997. Hybridization in the Sea. *In*: Blaxter, J.H.S. and Southward, A.J. (Eds.). *Advances in Marine Biology. Vol. 31*. New York: Academic Press, pp. 2-78.

Glaubitz, J.C. 2004. CONVERT: A User-friendly Program to Reformat Diploid Genotypic Data for Commonly Used Population Genetic Software Packages. *Molecular Ecology Notes*, 4: 309-310.

Gompert, Z. and Buerkle, C.A. 2013. Analyses of Genetic Ancestry Enable Key Insights for Molecular Ecology. *Mol. Ecol.*, 22: 5278-5294.

Gopurenko, D., Hughes, J.M. and Keenan, C.P. 1999. Mitochondrial DNA Evidence for Rapid Colonisation of the Indo-West Pacific by the Mud Crab *Scylla serrata*. *Mar. Biol.*, 134: 227-233.

Goudet, J. 1995. FSTAT version 1.2: A Computer Program to Calculate F-statistics. *Heredity*, 86(6): 485-486.

Grave, S.D., Pentcheff, N.D., Ahyong, S.T., Chan, T.Y., Crandall, K.A., Dworschak, P.C., Felder, D.L., Feldmann, R.M., Fransen, C.H.J.M., Goulding, L.Y.D., Lemaitre, R., Low, M.E.Y., Martin, J.W., Ng, P.K.L., Schweitzer, C.E., Tan, S.H., Tshudy D. and Wetzer, R. 2009. A Classification of Living and Fossil Genera of Decapod Crustaceans. *Raffles Bulletin of Zoology*, 21: 1-109.

Grewe, P.M., Krueger, C.C., Aquadro, C.F., Bermingham, E., Kincaid, H.L. and May, B. 1993. Mitochondrial DNA Variation among Lake Trout (*Salvenilus namaycush*) Strains Stocked into Lake Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, 50: 2397-2403.

Hall, A.T. 2007. BioEdit: A User-friendly Biological Sequence Alignment Editor and Program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.*, 41: 95-98.

Hanner, R., Corthals, A. and Dessauer, H.C. 2005. Salvage of Genetically Valuable Tissues Following a Freezer Failure. *Molecular Phylogenetics and Evolution*, 34: 452-455.

Hardy, O.J., Charbonnel, N., Freville, H. and Heuertz, M. 2003. Microsatellite Allele Sizes: A Simple Test to Assess Their Significance on Genetic Differentiation. *Genetics*, 163: 1467-1482.

Harrison, R.G. 1989. Animal Mitochondrial DNA as a Genetic Marker in Population and Evolutionary Biology. *Trends in Ecology and Evolution*, 4(1): 6-11.

He, G.H., Meng, R.H., Newman, M., Gao, G.Q., Pittman, R.N. and Prakash, C.S. 2003. Microsatellites as DNA Markers in Cultivated Peanut (*Arachis hypogaea* L.). *BMC Plant Biol.*, 3: 3.

Hebert, P.D.N., Ratnasingham, S. and DeWaard, J.R. 2003. Barcoding Animal Life: Cytochrome C Oxidase Subunit I Divergences among Closely Related Species. *Proc. R. Soc. Lond.*, 270: 96-99.

Hickerson, M.J., Meyer, C.P. and Moritz, C. 2006. DNA Barcoding Will Often Fail to Discover New Animal Species Over Broad Parameter Space. *Systematic Biology*, 55: 729-739.

Hidayat, T., Yukawa, T. and Ito, M. 2005. Molecular Phylogenetics of Subtribe Aeridinae (Orchidaceae): Insights from Plastid matK and Nuclear Ribosomal ITS Sequences. *J. Plant Res.*, 118: 271-284.

Hogg, I.D. and Hebert, P.D.N. 2004. Biological Identification of Springtails (Collembola: Hexapoda) from the Canadian Arctic, Using Mitochondrial DNA Barcodes. *Canadian Journal of Zoology*, 82: 749-754.

Holthuis, L.B. 2004. The Identity and Lectotype of *Portunus pelagicus* (L., 1758). *Crustaceana*, 77(10): 1267-1269.

Hudson, R.R., Slatkin, M. and Maddison, W.P. 1992. Estimation of Levels of Gene Flow from DNA Sequence Data. *Genetics*, 132: 583-589.

Ikhwanuddin, M., Azra, M.N., Sung, Y.Y., Bolong, A.M.A. and Long S. M. 2013. Growth and Survival of Blue Swimming Crab (*Portunus pelagicus*) Reared on Frozen and Artificial Foods. *Agricultural Sciences*, 4: 76-82.

Ikhwanuddin, M., Azra, M.N., Talpur, M.A.D., Abol-Munafi, A.B. and Shabdin M.L. 2012. Optimal Water Temperature and Salinity for Production of Blue Swimming Crab, *Portunus pelagicus* 1st Day Juvenile Crab. *AACL Bioflux*, 5(1): 4-8.

Ikhwanuddin, M., Liyana, A.N., Azra, M.N., Bachok, Z. and Abol-Munafi, A.B. 2014. Natural Diet of Blue Swimming Crab, *Portunus* pelagicus at Strait of Tebrau, Johor, Malaysia. *Sains Malaysiana*, 43(1): 37-44.

Imai, H., Fujii, Y., Karakawa, J., Yamamoto, S., Numachi, K. 1999. Analysis of the Population Structure of the Swimming Crab, *Portunus trituberculatus* in the Coastal Waters of Okayama Prefecture, by RFLPs in the Whole Region of Mitochondrial DNA. *Fish. Sci.*, 65: 655-656.

Inger, F.R. and Chin, P.K. 2002. *The Freshwater Fishes of North Borneo*. Kota Kinabalu: Natural History Publications.

Ingles, J.A. 2004. Status of the Blue Crab Fisheries in the Philippines. *In*: DA-BFAR (Department of Agriculture-Bureau of Fisheries and Aquatic Resources). *In Turbulent Seas: The Status of Philippine Marine Fisheries*. Coastal Resource Management Project, Cebu City, Philippines, pp. 47-52.

ITIS. *Integrated Taxonomic Information System: Portunus pelagicus (Linnaeus, 1758)*. Retrieved from http://www.itis.gov/servlet.

Johnston, D., Harris, D., Cauti, N. and Thomson, A. 2011. Decline of a Blue Swimmer Crab (*Portunus pelagicus*) Fishery in Western Australia- History, Contributing Factors and Future Management Strategy. *Fish. Res.*, 109: 119-130.

Josileen, J. 2011. Food and Feeding of the Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758) (Decapoda, Brachyura) along the Coast of Mandapam, Tamil Nadu, India. *Crustaceana*, 84(10): 1169-1180.

Jukes, T.H. and Cantor, C.R. 1969. Evolution of Protein Molecules. *In*: Munro, H.N. (Ed.). *Mammalian Protein Metabolism*. New York: Academic Press, pp. 21-132.

Jung, H., Lyons, R.E., Hurwood, D.A. and Mather, P.B. 2013. Genes and Growth Performance in Crustacean Species: A Review of Relevant Genomic Studies in Crustaceans and Other Taxa. *Reviews in Aquaculture*, 5: 77-110.

Kailola, P.J., Williams, M.J., Stewart, P.C., Reichelt, R.E., McNee, A. and Grieve, C. 1993. *Australian Fisheries Resources*. Australia: Bureau of Resource Sciences and Fisheries Research Development Corporation, pp. 422.

Kangas, M.I. 2000. Synopsis of the Biology and Exploitation of the Blue Swimmer Crab, *Portunus pelagicus* Linnaeus, in Western Australia. Fish. Res.Rep. *Fish. West. Aus.*, 121: 1-22.

Karl, S.A., Toonen, R.J., Grant, W.S. and Bowen, B.W. 2012. Common Misconceptions in Molecular Ecology: Echoes of the Modern Synthesis. *Mol. Ecol.*, 21: 4171-4189.

Kessing, B., Croom, H., Martin, A., McIntosh, C., McMillan, W.O. and Palumbi, S. 1989. *The Simple Fool's Guide to PCR version 1.0*. Department of Zoology, University of Hawaii, Honolulu.

Kimura, M. 1980. A Simple Method for Estimating Evolutionary Rates of Base Substitutions through Comparative Studies of Nucleotide Sequences. *J. Mol. Evol.*, 16: 111-120.

Kimura, M. and Crow, J.F. 1964. The Number of Alleles That Can Be Maintained in a Finite Populations. *Genetics*, 49: 725-738.

Kimura, M. and Otha, T. 1978. Stepwise Mutation Model and Distribution of Allelic Frequencies in a Finite Populations. *Proceedings of the National Academy of Sciences of the USA*, 75: 2868-2872.

Klangnurak, W., Phinchongsakuldit, J. and James, T. 2012. Population Structure and Genetic Connectivity of *Lutjanus russelli* (Lutjanidae) in Thailand. *Proceedings of the 12th International Coral Reef Symposium*. Cairns, Australia, July 9-13, 2012.

Klinbunga, S., Khetpu, K., Khamnamtong, B. and Menasveta, P. 2007. Development of a Species-specific SCAR Marker of the Blue Swimming Crab (*Portunus pelagicus*). *Biochem. Genet.*, 45: 755-760.

Klinbunga, S., Khetpu, K., Khamnamtong, B. and Menasveta, P. 2007. Genetic Heterogeneity of the Blue Swimming Crab (*Portunus pelagicus*) in Thailand Determined by AFLP Analysis. *Biochem. Genet.*, 45: 725-736.

Klinbunga, S., Thamniemdee, N., Yuvanatemiya, V., Khetpu, K., Khamnamtong, B. and Menasveta, P. 2010. Species Identification of the Blue Swimming Crab *Portunus pelagicus* in Thai Waters Using mtDNA and RAPD derived SCAR Markers. *Aquaculture*, 308: S39-S46.

Klinbunga, S., Yuvanatemiya, V., Wongphayak, S., Khetpu, K., Menasveta, P. and Khamnamtong, B. 2010. Genetic Population Differentiation of the Blue Swimming Crab *Portunus pelagicus* (Portunidae) in Thai Waters Revealed by RAPD Analysis. *Genet. Mol. Res.*, 9(3): 1615-1624.

Kocher, T.D. and Stepien, C.A. 1997. *Molecular Systematics of Fishes.* New York: Academic Press.

Kumar, S., Gupta, J., Kumar, N., Dikshit, K., Navani, N., Jain, P. and Nagarajan, M. 2006. Genetic Variation and Relationships among Eight Indian Riverine Buffalo Breed. *Molecular Ecology*, 15: 593-600.

Kumar, P., Gupta, V.K., Misra, A.K., Modi, D.R. and Pandey, B.K. 2009. Potential of Molecular Markers in Plant Biotechnology. *Plant Omics J.*, 2: 141-162.

Kunsook, C., Gajaseni, N. and Paphavasit, N. 2014. A Stock Assessement of the Blue Swimming Crab *Portunus pelagicus* (Linnaeus, 1758) for Sustainable Management in Kung Krabaen Bay, Gulf of Thailand. *Trop. Life Sci. Res.*, 25(1): 41-59.

Kunsook, C., Gajaseni, N. and Paphavasit, N. 2014. The Feeding Ecology of the Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758), at Kung Krabaen Bay, Chanthaburi Province, Thailand. *Trop. Life Sci. Res.*, 25(1): 13-27.

Lack, J.B., Pfau, R.S. and Wilson, G.M. 2010. Demographic History and Incomplete Lineage Sorting Obscure Population Genetic Structure of the Texas Mouse (*Peromyscus attwateri*). *Journal of Mammalogy*, 91(2): 314-325.

Lai, J.C.Y., Ng, P.K.L. and Davie, P.J.F. 2010. A Revision of the *Portunus pelagicus* (Linnaeus, 1758) Species Complex (Crustacea: Brachyura: Portunidae) with the Recognition of Four Species. *The Raffles Bulletin of Zoology*, 58(2): 199-237.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23(21): 2947-2948.

Lavery, S., Moritz, C., and Fielder, D.R. 1996. Indo-Pacific Population Structure and Evolutionary History of the Coconut Crab *Birgus latro*. *Molecular Ecology*, 5: 557-570.

Li, P.F., Liu, P., Li, J., Dai, F. and He, Y.Y. 2007. Biochemical Genetic Analysis of *Portunus trituberculatus* in Laizhou Bay. *Mar. Fish. Res.*, 28: 90-96.

Lian, C.L., Wadud, M.A., Geng, Q.F., Shimatani, K. and Hogetsu, T. 2006. An Improved Technique for Isolating Codominant Compound Microsatellite Markers. *J. Plant Res.*, 119: 415-417.

Liu, H. and Chen, Y. 2003. Phylogeny of the East Asian Cyprinids Inferred from Sequences of the Mitochondrial DNA Control Region. *Can. J. Zool.*, 81: 1938-1946.

Liu, Y., Liu R.L., Ye, L.C., Liang, J., Xuan, F.J. and Xu, Q.H. 2009. Genetic Differentiation between Populations of Swimming Crab *Portunus trituberculatus* along the Coastal Waters of the East China Sea. *Hydrobiologia*, 618: 125-137.

Liu, Y.G., Chen, S.L., Li, B.F., Wang, Z.J. and Liu, Z. 2005. Analysis of Genetic Variation in Selected Stocks of Hatchery Flounder, *Paralichthys olivaceus*, Using AFLP Markers. *Biochem. Syst. Ecol.*, 33: 993-1005.

Liu, Y.G., Guo, Y.H., Hao, J. and Liu, L.X. 2012. Genetic Diversity of Swimming Crab (*Portunus trituberculatus*) Populations from Shandong Peninsula as Assessed by Microsatellite Markers. *Biochem. Syst. Ecol.*, 41: 91-97.

Liu, Z.J. and Cordes, J.F. 2004. DNA Markers Technologies and Their Applications in Aquaculture Genetics. *Aquaculture*, 238: 1-37.

Ma, H.Y., Ma, C.Y. and Ma, L.B. 2011. Population Genetic Diversity of Mud Crab (*Scylla paramamosain*) in Hainan Island of China Based on Mitochondrial DNA. *Biochemical Systematics and Ecology*, 39: 434-440.

MacMillan, W.O., Raff, R.A. and Palumbi, S.R. 1992. Population Genetic Consequences of Developmental Evolution in Sea Urchins (genus *Heliocidaris*). *Evolution*, 46: 1299-1309.

Mantelatto, F.L., Robles, R. and Felder, D.L. 2007. Molecular Phylogeny of the Western Atlantic Species of the genus *Portunus* (Crustacea, Brachyura, Portunidae). *Zoological Journal of the Linnean Society*, 150: 211-220.

Mardis E.R. 2008. Next-generation DNA Sequencing Methods. *Annu. Rev. Genom. Hum. Genet.*, 9: 387-402.

Marshall, S., Warburton, K., Paterson, B. and Mann, D. 2005. Cannibalism in Juvenile Blue-swimmer Crabs *Portunus pelagicus* (Linnaeus, 1766): Effects of Body Size, Moult Stage and Refuge Availability. *Applied Animal Behaviour Science*, 90: 65-82.

Mat Jaafar, T.N.A., Taylor, M.I., Nor, S.A.M., Bruyn, M. and Carvalho, G.R. 2012. DNA Barcoding Reveals Cryptic Diversity within Commercially Exploited Indo-Malay Carangidae (Teleosteii: Perciformes). *PLOS ONE*, 7(11): e49623.

Maudet, C., Miller, C., Bassano, B., Breitenmoser-Wursten, C., Gauthier, D., Obexer-Ruff, G., Michallet, J., Taberlet, P. and Luikart, G. 2002. Microsatellite DNA and Recent Statistical Methods in Wildlife Conservation Management: Applications in Alpine Ibex [*Capra ibex (ibex)*]. *Molecular Ecology*, 11(3): 421-436.

McNeely, J.A., Miller, K.R., Reid, W.V., Mittermeier, R.A. and Werner, T.B. 1990. *Conserving the World's Biological Diversity.* World Conservation Union, World Resources Institute, Conservation International, World Wildlife Fund-US and the World Bank, Washington, D.C.

Melville-Smith, R., Bellchambers, L.M. and Kangas, M. 2001. *The Collection of Fisheries Data for the Management of the Blue Swimmer Crab Fishery in Central and Lower West Coasts of Australia*. FRDC Final Report Proj. No. 98/121, pp. 99.

Meyer, A. 1993. Evolution of Mitochondrial DNA in Fishes. *Biochemistry and Molecular Biology of Fishes*, 2: 1-38.

Moore, S.S., Sargeant, L.L., King, T.J., Mattick, J.S., Georges, M., Hetzel, D.J.S. 1991. The Conservation of Dinucleotide Microsatellites among Mammalian Genomes Allows the Use of Heterologous PCR Primer Pairs in Closely Related Species. *Genomics*, 10: 654-660.

Naim, D.M., Rosly, H.A.M. and Nor, S.A.M. 2012. Assessment of Phylogenetic Inter-Relationships in Mud Crab Genus *Scylla* (Portunidae) Based on Mitochondrial DNA Sequence. *Proceeding of International Conference on Applied Life Sciences (ICALS2012)*. Turkey, September 10-12, 2012, pp. 269-275.

Ng, P.K.L. 1998. Crabs. *In*: Carpenter, K.E. and Niem, V.H. (Eds.). *FAO Species Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific. Volume 2. Cephalopods, Crustaceans,* *Holothurians and Sharks*. Rome: Food and Agriculture Organisation, pp. 1045-1155.

Ng, P.K.L., Guinot, D. and Davie, P.J.F. 2008. Systema Brachyurorum: Part I. An Annotated Checklist of Extant Brachyuran Crabs of the World. *Raffles Bulletin of Zoology*, 17: 1-286.

Nguyen, T.T.T., Na-Nakorn, U., Sukmanomon, S. and Zi Ming, C. 2008. A Study on Phylogeny and Biogeography of Mahseer Species (Pisces: Cyprinidae) Using Sequences of Three Mitochondrial DNA Gene Regions. *Molecular Phylogenetics and Evolution*, 48(3): 1223-1231.

Nolan, D.V., Martin, S.A.M., Kelly, Y., Glennon, K., Palmer, R., Smith, T., McCormack, G.P. and Powell, R. 2000. Development of Microsatellite PCR Typing Methodology for the Sea Louse, *Lepeophtheirus salmonis* (Kroyer). *Aquac. Res.*, 31: 815-822.

Nyakaana, S., Tumusiime, C., Oguge, N., Siegismund, H.R., Arctander, P. and Muwanika, V. 2008. Mitochondrial DNA Diversity and Population Structure of a Forest-dependent Rodent, *Praomys taitae* (Rodentia: Muridae) Heller 1911, in the Fragmented Forest Patches of Taita Hills, Kenya. *South African Journal of Science*, 104: 499-504.

Oliveira, E.J., Padua, J.G., Zucchi, M.I., Vencovsky, R., Vieira, M.L.C. 2006. Origin, Evolution and Genome Distribution of Microsatellites. *Genet. Mol. Res.*, 29: 294-307.

Otto, R.S., Jamieson, G.S., Boutillier, T., Zhuang, Z.M., Hong, S.V., Armstrong, D.A., Sekiguchi, H., Ivanov, B.G., Rodin, V. and Yeon, I.J. 2001. *Commercially Important Crabs, Shrimps and Lobsters of the North Pacific Ocean*. National Pacific Marine Science Organisation (PICES) Report, 19: 1-74.

Ovenden, J.R., Brasher, D.J. and White, R.W.G. 1992. Mitochondrial DNA Analyses of the Red Rock Lobster *Jasus edwardsii* Supports an Apparent Absence of Population Subdivision throughout Australasia. *Mar. Biol.*, 112: 319-326.

Padate, V.P., Rivonker, C.U., Anil, A.C., Sawant, S.S. and Krishnamurthy, V. 2010. A New Species of Portunid Crab of the Genus *Charybdis* (De Haan, 1833) (Crustacea: Decapoda: Brachyura) from Goa, India. *Mar. Biol. Res.*, 6(6): 579-590.

Palumbi, S.R. and Wilson, A.C. 1990. Mitochondrial DNA Diversity in the Sea Urchins *Strongylogentrotus purpulatus* and *S. droebachiensis*. *Evolution*, 44: 403-415.

Palumbi, S.R., Martin, A., Romano, S., MacMillan, W.O., Stice, L. and Grabowski, G. 1991. *The Simple Fool's Guide to PCR, version 2.0*. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu.

Patel, N.M., Chaaya, N.D. and Bhaskaran, M. 1979. Stomach Content of *Portunus pelagicus* (Linn.) from AD Net Catches. *Indian Journal of Marine Science*, 8(1): 48-49.

Pfeiler, E., Hurtado, L.A., Knowles, L.L., Torre-Cosio, J., Bourillon-Moreno, L., Marquez-Farias, J.F. and Montemayor-Lopez, G. 2005. Population Genetics of the Swimming Crab *Callinectes bellicosus* (Brachyura: Portunidae) from the Eastern Pacific Ocean. *Mar. Biol.*, 146: 559-569.

Piepho, H.P. and Koch, G. 2000. Codominant Analysis of Banding Data from a Dominant Marker System by Normal Mixtures. *Genetics*, 155: 1459-1468.

Pillay, K.K. and Nair, J.B. 1976. Observations on the Breeding Biology of Some Crabs from the Southwest of India. *Journal of Marine Biological Association of India*, 15: 754-770.

Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L. and Estoup, A. 2004. GENECLASS2: A Software for Genetic Assignment and First-generation Migrant Detection. *Journal of Heredity*, 95(6): 536-539.

Piry, S., Luikart, G. and Cornuet, J.M. 1999. BOTTLENECK: A Computer Program for Detecting Recent Reductions in the Effective Population Size Using Allele Frequency Data. *Journal of Heredity*, 90(4): 502-503.

Place, A.R., Feng, X.J., Steven, C.R., Fourcade, H.M. and Boore, J.L. 2005. Genetic Markers in Blue Crabs (*Callinectes sapidus*) II. Complete Mitochondrial Genome Sequence and Characterization of Genetic Variation. *Journal of Experimental Marine Biology and Ecology*, 319: 15-27.

Potter, I.C., Chrystal, P.J. and Loneragan, N.R. 1983. The Biology of the Blue Manna Crab *Portunus pelagicus* in an Australian Estuary. *Mar. Biol.*, 78: 75-85.

Potter, I.C., de Lestang, S. and Young, G.C. 1998. *Influence of the Dawesville Channel on the Recruitment, Distribution and Emigration of Crustaceans and Fish in the Peel-Harvey Estuary*. FRDC Final Report Proj. No. 95/042, pp. 61.

Potter, M.A., Sumpton, W.D. and Smith, G.S. 1987. Queensland Sand Crab Study Highlights a Need for Changes in Management. *Australian Fisheries*, 46(6): 22-26.

Potter, M.A., Sumpton, W.D. and Smith, G.S. 1991. Movement, Fishing Sector Impact and Factors Affecting the Recapture Rate of Tagged Sand Crabs, *Portunus pelagicus* (L.), in Moreton Bay, Queensland. *Australian Journal of Marine and Freshwater Research*, 42: 751-760.

Pritchard, J.K., Stephens, M. and Donnelly, P. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, 155(2): 945-959.

Putman, A.I. and Carbone, I. 2014. Challenges in Analysis and Interpretation of Microsatellite Data for Population Genetic Studies. *Ecol. Evol.*, 4: 4399-4428.

Pyhajarvi, T., Garcia-Gil, M.R., Knurr, T., Mikkonen, M., Wachowiak, W. and Savolainen, O. 2007. Demographic History Has Influenced Nucleotide Diversity in European *Pinus sylvestris* Populations. *Genetics*, 177: 1713-1724.

Rannala, B. and Mountain, J.L. 1997. Detecting Immigration by Using Multilocus Genotypes. *Proceedings of the National Academy of Sciences of the United States of America*, 94(17): 9197-9201.

Raymond, M. and Rousset, F. 1995. GENEPOP: Population Genetic Software for Exact Test Ecumenicism. *Journal of Heredity*, 86: 248-249.

Reed, D.H. and Frankham, R. 2003. Correlation between Fitness and Genetic Diversity. *Conservation Biology*, 17(1): 230-237.

Refaat, A.M. 2012. *Applications of DNA Fingerprinting in Solving Forensic Cases*. Naif Arab University for Security Sciences.

Reynolds, J.D., Mace, G.M., Redford, K.H. and Robinson, J.G. 2001. *Conservation of Exploited Species*. UK: Cambridge University Press.

Rogers, A.R. and Harpending, H. 1992. Population Growth Makes Waves in the Distribution of Pairwise Genetic Differences. *Molecular Biology and Evolution*, 9(3): 552-569.

Romano, N. and Zeng, C. 2008. Blue Swimmer Crabs: Emerging Species in Asia. *Global Aquaculture Advocate*, pp. 34-36.

Rosly, H.A.M., Nor, S.A.M., Yahya, K. and Naim, D.M. 2013. Mitochondrial DNA Diversity of Mud Crab *Scylla olivacea* (Portunidae) in Peninsular Malaysia: A Preliminary Assessment. *Mol. Biol. Rep.*, 40: 6407-6418.

Rousset, F. 2008. Genepop'007: A Complete Reimplementation of the Genepop Software for Windows and Linux. *Mol. Ecol. Resources*, 8: 103-106.

Rozas, J., Sanchez-Delbarrio, J.C., Messeguer, X. and Rozas, R. 2003. DnaSP, DNA Polymorphism Analyses by the Coalescent and Other Methods. *Bioinformatics*, 19: 2496-2497.

Rubinoff, D. 2006. Utility of Mitochondrial DNA Barcodes in Species Conservation. *Conservation Biology*, 20(4): 1026-1033.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. 1988. Primer-directed Amplification of DNA with a Thermostable DNA Polymerase. *Science*, 239: 487-491.

Saitou, N. and Nei, M. 1987. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. *Mol. Biol. Evol.*, 4(4): 406-425.

Sanger, F., Nicklen, S. and Coulson, A.R. 1977. DNA Sequencing with Chain-terminating Inhibitors. *Proc. Natl. Acad. Sci. U.S.A.*, 74: 5463-5467.

Sanvicente-Anorve, L., Gomez-Ponce, A., Vazquez-Bader, A.R. and Gracia, A. 2008. Morphometry and Relative Growth of the Swimming Crab, *Portunus spinicarpus* (Stimpson, 1871) from the Southern Gulf of Mexico. *Crustaceana*, 81(3): 329-339.

Saunders, N.C., Kessler, L.G. and Avise, J.C. 1986. Genetic Variation and Geographic Differentiation in Mitochondrial DNA of the Horseshoe Crab, *Limulus polyphemus*. *Genetics*, 112: 613-627.

Schlotterer, C., Amos, B. and Tautz, D. 1991. Conservation of Polymorphic Simple Sequences in Cetacean Species. *Nature*, 354: 63-65.

Schubart, C.D. 2001. Lack of Divergence between 16S mtDNA Sequences of the Swimming Crabs *Callinectes bocourti* and *C. maracaiboensis* (Brachyura: Portunidae) from Venezuela. *Fish. Bull.*, 99: 475-481.

Scribner, K.T., Gust, J.R. and Field, R.L. 1996. Isolation and Characterization of Novel Salmon Microsatellite Loci: Cross Species Amplification and Population Genetics Application. *Can. J. Fish. Aquatic Science*, 53: 833-841.

Scribner, K.T., Page, K.S. and Bartron, M.L. 2000. Hybridization in Freshwater Fishes: A Review of Case Studies and Cytonuclear Methods of Biological Inference. *Reviews in Fish Biology and Fisheries*, 10: 293-323.

Selkoe, K.A. and Toonen, R.J. 2006. Microsatellites for Ecologists: A Practical Guide to Using and Evaluating Microsatellite Markers. *Ecol. Lett.*, 9: 615-629.

Senan, S., Kizhakayil, D., Sasikumar, B. and Sheeja, T.E. 2014. Methods for Development of Microsatellite Markers: An Overview. *Not. Sci. Biol.*, 6: 1-13.

Sezmis, E. 2004. *The Population Genetic Structure of Portunus pelagicus in Australian Waters*. PhD Thesis, Murdoch University.

Shearer, T.L., Van Oppen, M.J.H., Romano, S.L. and Worheide, G. 2002. Slow Mitochondrial DNA Sequence Evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, 11: 2475-2487.

Shinkarenko, L., 1979. Development of the Larval Stages of the Blue Swimmer Crab *Portunus pelagicus* L. (Portunidae: Decapoda: Crustacea). *Aust. J.Mar. Freshw. Res.*, 30: 485-503.

Sienes, P.M.Q., Willette, D.A., Romena, L.R., Alvior, C.G. and Janet, S.E. 2014. Genetic Diversity and the Discovery of a Putative Cryptic Species within a Valued Crab Fishery, *Portunus pelagicus* (Linnaeus 1758) in the Philippines. *Philippine Science*, 7(2): 317-323.

Sindermann, C.J. 2006. *Coastal Pollution: Effects on Living Resources and Humans*. US: CRP Press.

Sleator, R.D. 2010. An Overview of the Processes Shaping Protein Evolution. *Sci. Prog.*, 93: 1-6.

Sleator, R.D. 2011. Phylogenetics. *Arch. Microbiol.*, 193: 235-239.

Smith, H. 1982. Blue Crabs in South Australia- Their Status, Potential and Biology. *SAFIC*, 6(5): 6-9.

Stephenson, W. 1962. Evolution and Ecology of Portunid Crabs, with Especial Reference to Australian Species. *In*: Leeper, G.W. (Ed.). *The Evolution of Living Organisms*. Melbourne: Melbourne University Press, pp. 311-327.

Sultmann, H. and Mayer, W.E. 1997. Reconstruction of Cichlid Phylogeny Using Nuclear DNA Markers. *In*: Kocher, T.D. and Stephien, C.A. (Eds.). *Molecular Systematics of Fishes*. San Diego: Academic Press, pp. 39-51.

Sumner, N.R., Malseed, B.E. and Williamson, P.C. 2000. *Estimating the Recreational Catch of Blue Swimmer Crab in the South West of Western Australia*. FRDC Report 98/119.

Svane, I. and Hooper, G. 2004. *Blue Swimmer Crab (Portunus pelagicus) Fishery. Fishery Assessment Report to PIRSA for the Blue Crab Fishery Management Committee*. Australia: South Australian Research and Development Institute, Government of South Australia.

Tajima, F. 1989. Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. *Genetics*, 123: 585-595.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) Software version 4.0. *Molecular Biology and Evolution*, 24(8): 1596-1599.

Tanner, J.E. 2007. Influence of Introduced European Green Crabs (*Carcinus maenas*) on Habitat Selection by Juvenile Native Blue Crabs (*Portunus pelagicus*). *Estuaries and Coasts*, 30(4): 601-606.

Tateno, Y., Takezaki, N. and Nei, M. 1994. Relative Efficiencies of the Maximum Likelihood, Neighbor Joining and Maximum Parsimony Methods When Substitution Rate Varies with Site. *Molecular Biology and Evolution*, 11: 261-277.

Taylor, B. 2013. *Blue and Red Swimmer Crab Seafood Watch Report*. Monterey Bay Aquarium.

Temnykh, S., DeClerck, G., Lukashova, A., Lipovich, L., Cartinhour, S. and McCouch, S. 2001. Computational and Experimental Analysis of Microsatellites in Rice (*Oryza sativa* L.): Frequency, Length Variation, Transposon Associations and Genetic Marker Potential. *Genome Res.*, 11: 1441-1452.

Todd, R., Donoff, R.B., Kim, Y. and Wong, D.T. 2001. From the Chromosome to DNA: Restriction Fragment Length Polymorphism Analysis and Its Clinical Application. *J. Oral Maxillofac. Surg.*, 59: 660-667.

Toro, M.A. and Caballero A. 2005. Characterization and Conservation of Genetic Diversity in Subdivided Populations. *Phil. Trans. R. Soc. B.*, 360: 1367-1378.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. and Shipley, P. 2004. MICRO-CHECKER: Software for Identifying and Correcting Genotyping Errors in Microsatellite Data. *Molecular Ecology Notes*, 4(3): 535-538.

Vartak, V.R., Narasimmalu, R., Annam, P.K., Singh, D.P. and Lakra, W.S. 2015. DNA Barcoding Detected Improper Labelling and Supersession of Crab Food Served by Restaurants in India. *J. Sci. Food Agric.*, 95: 359-366.

Vaughn, K.C., DeBonte, L.R. and Wilson, K.G. 1980. Organelle Alteration as a Mechanism for Maternal Inheritance. *Science*, 208: 196-197.

Villesen, P. 2007. *FaBox: An Online Fasta Sequence Toolbox*. Retrieved from http://www.birc.au.dk/software/fabox.

Vorburger, C. and Ribi, G. 1999. Aggression and Competition for Shelter between a Native and an Introduced Crayfish in Europe. *Freshwater Biology*, 42: 111-119.

Voris, H.K. 2000. Maps of Pleistocene Sea Levels in Southeast Asia: Shorelines, River Systems and Time Durations. *Journal of Biogeography*, 27: 1153-1167.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: A New Technique for DNA Fingerprinting. *Nucleic Acids Res.*, 23: 4407-4414.

Vrijenhoek, R.C. 1998. Conservation Genetics of Freshwater Fish. *Journal of Fish Biology*, 53: 394-412.

Wahlund, S. 1928. The Combination of Populations and the Appearance of Correlation Examined from the Standpoint of the Study of Heredity. *Hered.*, 11: 65-106.

Wang, C., Yu, X. and Tong, J. 2007. Microsatellite Diversity and Population Genetic Structure of Redfin Culter (*Culter erythropterus*) in Fragmented lakes of the Yangtze River. *Hydrobiologia*, 586(1): 321-329.

Wang, G.L., Jin, S., Li, Z. and Chen, Y.E. 2005. Tissue Specificity and Biochemical Genetic Analysis of Isozyme on Cultured *Portunus trituberculatus* Stock. *Journal of Oceanography in Taiwan Strait*, 24: 474-480.

Ward, R.D., Costa, F.O., Holmes, B.H. and Steinke, D. 2008c. DNA Barcoding Shared Fish Species from the North Atlantic and Australasia: Minimal Divergence for Most Taxa but a Likely Two Species for Both *Zeus faber* (John Dory) and *Lepidopus caudatus* (Silver Scabbardfish). *Aquatic Biology*, 3: 71-78.

Ward, R.D., Hanner, R. and Hebert, P.D.N. 2009. The Campaign to DNA Barcode All Fishes, FISH-BOL. *Journal of Fish Biology*, 74: 329-356.

Ward, R.D., Holmes, B.H. and Yearsley, G.K. 2008a. DNA Barcoding Reveals a Likely Second Species of Asian Seabass (Barramundi) (*Lates calcarifer*). *Journal of Fish Biology*, 72: 458-463.

Ward, R.D., Holmes, B.H., White, W.T. and Last, P.R. 2008b. DNA Barcoding Australian Chondrichthyans: Results and Potential Uses in Conservation. *Marine and Freshwater Research*, 59: 57-71.

Ward, R.D., Woodwark, M. and Skibinski, D.O.F. 1994. A Comparison of Genetic Diversity Levels in Marine, Freshwater and Anadromous Fish. *Journal of Fish Biology*, 44: 213-232.

Waters, J.M. and Wallis, G.P. 2000. Across the Southern Alps by River Capture? Freshwater Fish Phylogeography in South Island, New Zealand. *Mol. Ecol.*, 9: 1577-1582.

Weir, B.S. and Cockerham, C.C. 1984. Estimating F-statistics for the Analysis of Population Structure. *Evolution*, 38(6): 1358-1370.

Wickins, J.F. and Lee, D.O. 2002. *Crustacean Farming: Ranching and Culture*. Oxford: Blackwell Scientific Publications.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA Polymorphisms Amplified by Arbitrary Primers are Useful as Genetic Markers. *Nucleic Acids Res.*, 18: 6531-6535.

Williams, M.J. 1982. Natural Food and Feeding in the Commercial Sand Crab *Portunus pelagicus* Linnaeus, 1766 (Crustacea: Decapoda: Portunidae) in Moreton Bay, Queensland. *Journal of Experimental Marine Biology and Ecology*, 59: 165-176.

Wu, R.S.S. and Shin, P.K.S. 1998. Food Segregation in Three Species of Portunid Crabs. *Hydrobiologia*, 362(1): 107-113.

Xu, Q.H. and Liu, R. 2011. Development and Characterization of Microsatellite Markers for Genetic Analysis of the Swimming Crab, *Portunus trituberculatus*. *Biochem. Genet.*, 49: 202-212.

Xu, Q.H., Liu, R.L. and Liu, Y. 2009. Genetic Population Structure of the Swimming Crab, *Portunus trituberculatus* in the East China Sea Based on mtDNA 16S rRNA Sequences. *J. Exp. Mar. Biol. Ecol.*, 371: 121-129.

Yamauchi, M.M., Miya, M.U., Nishida, M. 2003. Complete Mitochondrial DNA Sequence of the Swimming Crab, *Portunus trituberculatus* (Crustacea: Decapoda: Brachyura). *Gene*, 311: 129-135.

Yap, E.S., Sezmis, E., Chaplin, J.A., Potter, I.C. and Spencer, P.B.S. 2002. Isolation and Characterization of Microsatellite Loci in *Portunus pelagicus* (Crustacea: Portunidae). *Mol. Ecol. Notes*, 2: 30-32.

Yednock, B.K. and Niegel, J.E. 2014. An Investigation of Genetic Population Structure in Blue Crabs, *Callinectes sapidus*, Using Nuclear Gene Sequences. *Mar. Biol.*, 161: 871-886.

Yu, C.G., Song, H.T. and Yao, G.Z. 2004. Assessment of the Crab Stock Biomass in the Continental Shelf Waters of the East China Sea. *J. Fish. Chin.*, 28: 41-46.

Yue, G.H., Li, Y., Lim, L.C. and Orban, L. 2004. Monitoring the Genetic Diversity of Three Asian Arowana (*Scleropages formosus*) Captive Stocks Using AFLP and Microsatellites. *Aquaculture*, 237: 89-102.

Zane, L., Bargelloni, L. and Patarnello, T. 2002. Strategies for Microsatellite Isolation: A Review. *Molecular Ecology*, 11: 1-16.

Zardoya, R., Vollmer, D.M., Craddock, C., Streetman, J.T., Karl, S. and Meyer, A. 1996. Evolutionary Conservation of Microsatellite Flanking Regions and Their Use in Resolving the Phylogeny of Cichlid Fishes (Pisces: Perciformes). *Proceedings of the Royal Society of London B*, 263: 1589-1598.