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
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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
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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
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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 didapat di kalangan populasi P. pelagicus dengan kelpelbagaian haplotaip yang tinggi dan kelpelbagaian 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 penghijrahkan 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 didapat 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. Walau bagaimanapun, 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 Fadhl 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 Sukimim 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.

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<td>°C</td>
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<td>m</td>
<td>Meter</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
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<td>Microliter</td>
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<td>bp</td>
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<td>Kilobase</td>
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<td>Tris/Borate/EDTA</td>
</tr>
<tr>
<td>LE</td>
<td>Low electroendosmosis</td>
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<tr>
<td>HR</td>
<td>High resolution</td>
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<tr>
<td>ATL</td>
<td>Animal tissue lysis</td>
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<tr>
<td>AL</td>
<td>Animal lysis</td>
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<td>AW</td>
<td>Animal wash</td>
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<tr>
<td>AE</td>
<td>Animal elution</td>
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<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
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<tr>
<td>CI</td>
<td>Consistency index</td>
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<td>RI</td>
<td>Retention index</td>
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<td>HI</td>
<td>Homoplasy index</td>
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<tr>
<td>ML</td>
<td>Maximum Likelihood</td>
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<td>MP</td>
<td>Maximum Parsimony</td>
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<tr>
<td>NJ</td>
<td>Neighbour Joining</td>
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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. sanguinoventus* 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).
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