UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT, CHARACTERIZATION AND APPLICATIONOF MICROSATELLITE MARKERS IN POPULATION GENETICS OF THE HORSESHOE CRAB, *Tachypleus gigas* MULLER OF PENINSULAR MALAYSIA

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By

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

DEVELOPMENT, CHARACTERIZATION AND APPLICATION OF MICROSATELLITE MARKERS IN POPULATION GENETICS OF THE HORSESHOE CRAB, *Tachypleus gigas* MULLER, OF PENINSULAR MALAYSIA

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Faculty: Biotechnology and Biomolecular Sciences

A total of 136 microsatellite sequences were isolated from *Tachypleus gigas* by using the 5' anchored PCR technique and 52 primer pairs were designed to flank these repeat regions. Of these, 18 primer pairs were found to be polymorphic and were used to examine the levels of genetic variation for 130 individuals of *T. gigas* from five populations (Port Dickson, Pantai Bersih, Sungai Muar, Pantai Balok and Kampung Sungai Pulai) collected along the coastal areas of Peninsular Malaysia. The 18 loci generated 51 alleles and the number of alleles per locus ranged from 2 to 6 (average 2.8 alleles per locus) with the mean observed heterozygosities ranging from 0.2590 to 0.3637. All the five populations showed heterozygote deficiencies. Inbreeding, small population size and the presence of null alleles may have contributed to the occurrence of heterozygote deficiency. Cluster analysis revealed

that the east coast population (Pantai Balok) was outgrouped from the west coast populations (Port Dickson, Pantai Bersih, Sungai Muar and Kampung Sungai Pulai). This result was in contrast to the Inter Simple Sequence Repeat (ISSR) cluster analysis where the populations were not differentiated in accordance with their geographical distributions by using five ISSR primers which generated 56 polymorphic loci. The possible reasons for were the low number of polymorphic loci used and the limitations of the ISSR technique such as dominant inheritance. In addition, the genetic differences between the polluted population, Port Dickson and the other populations suggested the possible use of *T. gigas* as a pollution bioindicator based on ISSR markers. The present results obtained from both the microsatellite and ISSR methods provide guidance for their future efficient use in the genetic analysis of horseshoe crabs. The microsatellite markers developed in this study will be useful for investigating the genetic diversity and population structure of *T. gigas* in the Southeast Asian region.

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

PEMBANGUNAN, PENCIRIAN DAN APPLIKASI PENANDA MIKROSATELIT DALAM GENETIK POPULASI BAGI BELANGKAS, *Tachypleus gigas* MULLER, SEMENANJUNG MALAYSIA

Oleh

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Sebanyak 136 urutan mikrosatelit telah dipencilkan daripada *Tachypleus gigas* dengan menggunakan teknik *5' anchored PCR* dan 52 pasangan primer telah direka. Daripada jumlah ini, 18 pasangan primer didapati polimorfik dan telah digunakan untuk menilai tahap variasi genetik untuk 130 individu *T. gigas* dari lima populasi (Port Dickson, Pantai Bersih, Sungai Muar, Pantai Balok and Kampung Sungai Pulai) yang dikutip di sepanjang kawasan pantai Semenanjung Malaysia. Lapan belas lokus menghasilkan 51 alel dengan bilangan alel per lokus berjulat antara 2 hingga 6 (purata 2.8 alel per lokus) dan min heterozigositi cerapan berjulat antara 0.2590 hingga 0.3637. Kesemua lima populasi menunjukkan kekurangan heterozigositi. Pembakaan tunggal, populasi saiz kecil dan kehadiran *null allele* mungkin menyumbang kepada berlakunya kekurangan heterozigositi. Analisis kelompok

menunjukkan bahawa populasi Pantai Timur (Pantai Balok) diasingkan daripada kumpulan populasi Pantai Barat (Port Dickson, Pantai Bersih, Sungai Muar dan Kampung Sungai Pulai). Keputusan ini adalah berbeza dengan analisis kelompok *Inter Simple Sequence Repeat* (ISSR) di mana populasi tidak dibezakan mengikut taburan geografi mereka dengan menggunakan lima primer ISSR yang menghasilkan 56 lokus polimorfik. Sebab-sebab yang mungkin untuk adalah bilangan rendah lokus polimorfik yang digunakan dan batasan teknik ISSR seperti warisan dominan. Di samping itu, perbezaan genetik antara populasi yang tercemar, Port Dickson dengan populasi lain mencadangkan penggunaan mungkin *T. gigas* sebagai petunjuk biologi pencemaran berdasarkan penanda ISSR. Keputusan yang diperolehi daripada kedua-dua kaedah mikrosatelit dan ISSR memberikan panduan untuk kegunaan masa depan dalam analisis genetik bagi belangkas. Penanda mikrosatelit yang dibangunkan dalam kajian ini akan menjadi berguna bagi menyiasat kepelbagaian genetik dan struktur populasi *T. gigas* di rantau Asia Tenggara.

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I certify that a Thesis Examination Committee has met on 18th April 2012 to conduct the final examination of Liew Pui Ling on her thesis entitled "Development, Characterization and Application of Microsatellite Markers in Population Genetics of the Horseshoe Crab, *Tachypleus gigas* Muller, of Peninsular Malaysia" 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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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

LIEW PUI LING

Date: 18 April 2012

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