



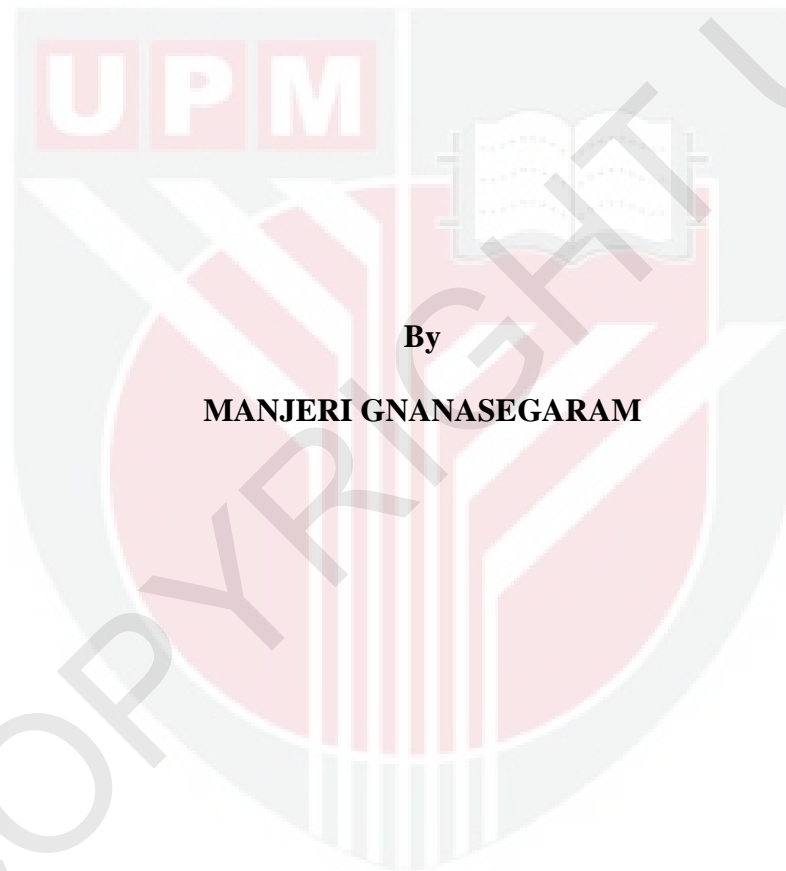
**UNIVERSITI PUTRA MALAYSIA**

***MORPHOMETRIC AND MOLECULAR GENETIC STUDIES ON  
RHINOCEROS BEETLE (ORYCTES RHINOCEROS LINNAEUS)  
POPULATIONS IN OIL PALM PLANTATIONS***

**MANJERI GNANASEGARAM**

**FP 2013 7**

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RHINOCEROS BEETLE (*ORYCTES RHINOCEROS* LINNAEUS)  
POPULATIONS IN OIL PALM PLANTATIONS**



**By**

**MANJERI GNANASEGARAM**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
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**January 2013**

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**MANJERI GNANASEGARAM**

**January 2013**

**Chairperson : Professor Rita Muhamad, PhD**

**Faculty : Agriculture**

*Oryctes rhinoceros*, commonly known as the rhinoceros beetle is an important agricultural pest that is known to inflict serious damage on young oil palm trees. Control and trapping procedures in plantations nationwide commonly incorporate the usage of pheromone lures. However, beetles were widely assumed to exhibit selective attraction level towards the pheromone lures. This initiated the interest to study the population structure of *O. rhinoceros* via morphometric and population genetic studies to assess the possible occurrence of a cryptic complex. Samples of *O. rhinoceros* beetles were collected from young oil palm replanting sites at Felcra Berhad, Perak, Tennamaram Estate; Selangor, Kuantan Trading Plantation; Pahang and Paya Pinang Plantation; Medan using a light trap and also a pheromone trap at each site. A working hypothesis is that beetles trapped by each procedure correspond, respectively, to different populations. Morphometric studies were carried out based on the measurements of total body length, elytron length, width of pronotum, length of pronotum and length of cephalic horn. Based on this study, individuals from the six populations were observed to overlap with one another in

the scatter plot produced by the principal component analysis and the canonical discriminant analysis, highlighting that the populations are morphologically indistinguishable. Meanwhile, population genetic studies were carried out using random amplified microsatellite (RAM) markers and also single locus deoxyribonucleic acid (DNA) microsatellite markers. In the RAMs study, a total of 78 reproducible, polymorphic loci were generated using seven RAMs primers. Similarity in geographical location, as well as the possible occurrence of two groups in *O. Rhinoceros* (the putative pheromone and light group) could have influenced the clustering pattern of the populations. However, the clustering did not diagnostically group any *O. rhinoceros* population to provide a clear evidence of a cryptic species complex. Next, using the 5' anchored random amplified microsatellites-polymerase chain reaction (RAMs-PCR) technique, a total of 180 microsatellite repeat motifs were isolated and a total of 144 primer pairs were designed. The isolated repeat motifs consisted of 151 perfect repeats, 14 interrupted repeats, 10 compound repeats, three interrupted compound repeats, one interrupted complex repeat and one complex repeat. After thorough optimizations of the developed markers, a total of 30 polymorphic single locus DNA microsatellite markers were identified and used to screen through the six populations of *O. rhinoceros*. Using these microsatellite markers, a total of 84 alleles were successfully amplified with molecular weights ranging from 113 bp to 328 bp. The number of alleles per locus ranged from two to eight alleles. The observed heterozygosities ranged from 0.0335 to 0.9333. The polymorphic information content (PIC) for the 30 loci ranged from 0.0478 to 0.7461. Further analysis on the variations within and between populations indicated a randomly mating population with free exchange of genes. Variations within populations were higher than between populations. The clustering pattern of the

populations was observed to be influenced by similarity in geographical location and possible events of migration. In this study, the population structure of the *O. rhinoceros* beetles has been exhaustively studied based on morphometric analysis and also molecular markers. It has been ascertained that there is no possible cryptic complex occurring in the *O. rhinoceros* populations studied.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGAJIAN MORFOMETRIK DAN GENETIK MOLEKULAR KE ATAS  
POPULASI KUMBANG BADAK (*ORYCTES RHINOCEROS* LINNAEUS) DI  
DALAM LADANG KELAPA SAWIT**

Oleh

**MANJERI GNANASEGARAM**

**January 2013**

**Pengerusi : Professor Rita Muhamad, PhD**

**Fakulti : Pertanian**

Kumbang *Oryctes rhinoceros* yang secara kebiasaannya dikenali sebagai kumbang badak merupakan perosak tanaman yang diketahui mampu menyebabkan kerosakan yang serius ke atas tanaman kelapa sawit muda. Kaedah pengawalan dan pemerangkapan yang kebiasaannya diamalkan di ladang adalah berdasarkan kepada perangkap feromon. Walaubagaimanapun, populasi kumbang dipercayai mempamerkan tarikan yang berlainan terhadap perangkap feromon. Situasi ini menjana keinginan untuk mendalami struktur populasi *O. rhinoceros* melalui kajian morfometrik dan kajian genetik populasi bagi mengenalpasti kebarangkalian kewujudan spesis kriptik. Sampel kumbang *O. rhinoceros* ditangkap dari tapak penanaman kelapa sawit muda di Felcra Berhad, Perak, Tennamaram Estate; Selangor, Kuantan Trading Plantation; Pahang dan Paya Pinang Plantation; Medan dengan menggunakan perangkap cahaya dan perangkap feromon di setiap lokasi. Kumbang yang ditangkap dengan kaedah yang berlainan diklasifikasikan sebagai populasi yang berlainan. Kajian morfometrik telah dilaksanakan berdasarkan pada ukuran panjang badan, panjang elytron, lebar pronotum, panjang pronotum dan

panjang tanduk. Berdasarkan kajian ini, individu daripada enam populasi yang dikaji diperhatikan bertindih antara satu sama lain dalam graf berselerak yang dihasilkan melalui analisis komponen utama dan analisis diskriminan. Keadaan ini menunjukkan bahawa populasi *O. rhinoceros* tidak dapat dibezakan menerusi diskriminasi morphometrik. Sementara itu, kajian populasi genetik telah dijalankan dengan menggunakan penanda mikrosatelit rawak dan penanda mikrosatelit DNA satu lokus. Kajian penanda mikrosatelit rawak telah menghasilkan 78 lokus polimorfik dengan menggunakan tujuh primer mikrosatelit rawak. Dalam kajian ini, pengkelompokan populasi kumbang dipercayai dipengaruhi oleh persamaan dari segi kedudukan geografi. Selain itu, terdapat kemungkinan wujudnya dua kumpulan kumbang iaitu kumpulan pheromone dan kumpulan cahaya berdasarkan perbezaan pengkelompokan kedua-dua populasi dari negeri Perak. Walaubagaimanapun, disebabkan tiada pengkelompokan individu secara diagnostik, maka kewujudan spesis kriptik tidak dapat dipastikan. Seterusnya, menerusi teknik “5’ anchored randomly amplified microsatellite-polymerase chain reaction (RAMs-PCR)” sejumlah 180 jujukan mikrosatelit telah berjaya dikenalpasti dan sejumlah 144 pasang primer mikrosatelit telah direka. Jujukan mikrosatelit yang telah berjaya dikenalpasti terdiri daripada 151 mikrosatelit sempurna, 14 mikrosatelit terganggu, 10 mikrosatelit sebatian, tiga mikrosatelit sebatian terganggu, satu mikrosatelit kompleks terganggu dan satu mikrosatelit kompleks. Selepas melalui proses pengoptimasian, sebanyak 30 mikrosatelit polimorfik satu lokus telah dikenal pasti dan digunakan untuk menganalisis enam populasi *O. rhinoceros*. Berdasarkan analisis yang dijalankan, sebanyak 84 alel telah berjaya dikesan dan ia berada di antara berat molekul 113 bp dan 328 bp. Bilangan alel bagi satu locus adalah dalam lingkungan dua hingga lapan alel. “Heterozygosities” diperhatikan berada antara

0.0335-0.9333. Kandungan maklumat polimorfik (PIC) bagi 30 lokus yang di kaji adalah antara 0.047814 dan 0.746129. Melalui penganalisan statistik, didapati populasi *O. rhinoceros* mengawan secara rawak dan ini menghasilkan saling pertukaran gen antara populasi. Variasi di dalam populasi adalah lebih tinggi daripada di antara populasi kumbang. Corak pengkelompokkan populasi kumbang dipengaruhi oleh persamaan lokasi geografi dan aktiviti migrasi. Secara kesimpulannya, kajian ini telah berjaya mendalami struktur populasi kumbang *O. rhinoceros* berdasarkan kajian morfometrik dan penanda molekular. Ia telah dapat dipastikan bahawa spesis kriptik tidak wujud dalam populasi *O. rhinoceros*.



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I certify that a Thesis Examination Committee has met on the **7th January 2013** to conduct the final examination of **Manjeri Gnanasegaram** on her thesis entitled "**MORPHOMETRIC AND MOLECULAR GENETIC STUDIES ON RHINOCEROS BEETLE (*Oryctes rhinoceros* Linnaeus) POPULATIONS IN OIL PALM PLANTATIONS**" 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 **Doctor of Philosophy**.

Members of the Thesis Examination Committee were as follows:

**Kamaruzzaman b Sijam, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Tan Wen Siang, PhD**

Professor  
Faculty of Biotechnology and Biomolecular  
Universiti Putra Malaysia  
(Internal Examiner)

**Nur Azura binti Adam, PhD**

Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**David Edward Bignell, PhD**

Professor  
The School of Biological and Chemical Sciences  
Queen Mary, University of London  
United Kingdom  
(External Examiner)

---

**SEOW HENG FONG, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

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

**Rita Muhamad Awang, PhD**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Tan Soon Guan, PhD**

Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

**Faridah binti Qamaruz Zaman, PhD**

Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

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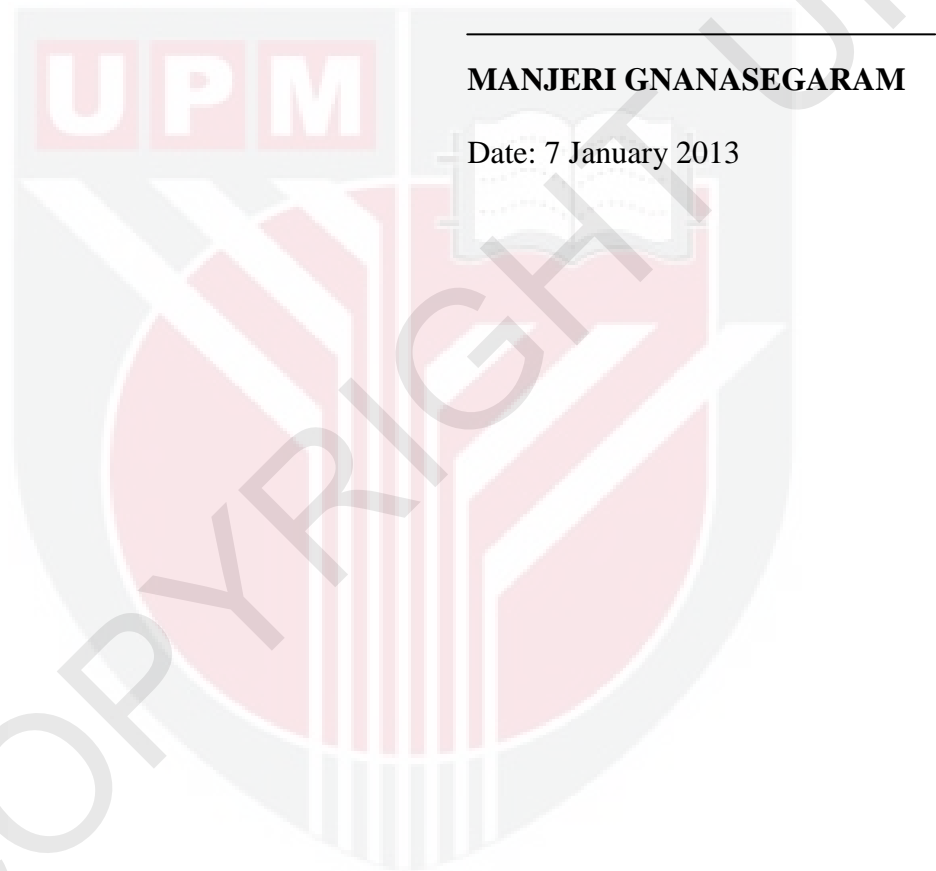
**BUJANG KIM HUAT, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## 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.



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