UNIVERSITI PUTRA MALAYSIA

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EPHIPPIA PRODUCTION AND GENETIC BIODIVERSITY IN TROPICAL CLADOCERAN *Moina micrura* Kurz

By

MOHD AZURAIDI BIN OSMAN

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

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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

EPHIPPIA PRODUCTION AND GENETIC BIODIVERSITY IN TROPICAL CLADOCERAN *Moina micrura* Kurz

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Chairperson: Professor Mariana Nor Shamsudin, PhD

Institute : Institute of Bioscience

In Malaysia, the imported *Artemia* nauplii is a standard larval diet in aquaculture and hatchery production system due to its availability in dormant form. However, this imported live feed is expensive and they can only survive for few hours in freshwater. Therefore, an easily available inexpensive local live-feed alternative for this imported species is desirable. In this study, the effect of different food concentrations and feeding frequencies on the production of ephippia had been investigated. Different food concentrations consisting of *Nannochloropsis oculata* ($4x10^6$ cells/ml, $4x10^4$ cells/ml, $4x10^2$ cells/ml, no food supply as control) and different feeding frequencies (3 days, 3 and 5 days, 5 days, 8 days, 8 and 3 days and daily) were used. The highest mean number of ephippia (7.33±1.53 ephippia) was produced when the cultures were fed every 3 and 5 days alternately. When the cultures were fed with $4x10^6$ cells/ml, no ephippia was produced due to the high amount of food available. In another set of experiment, parthenogenetic reproduction,

appearance of males and production of ephippia in *M. micrura* were studied. Two hundreds female individuals aged less than 24 hours were used. The cultures were fed with *N. oculata* at different food concentrations ($4x10^6$ cells/ml, $4x10^4$ cells/ml, $4x10^2$ cells/ml, no food supply as control) for 12 days. The highest number of males (186.7±23.1 males/l) was produced in the cultures fed with $4x10^2$ cells/ml of *N. oculata* when population density reached > 1600 individuals/l. Similarly, the highest total mean number of ephippia (160.0±0.0 ephippia/l) was achieved with *M. micrura* fed with $4x10^2$ cells/ml of *N. oculata* when the population density reached > 2000 individuals/l. Combination of limited food supply and high population density were needed to trigger the ephippia production. The hatchability of the ephippia that were produced in the earlier experiments was studied. In this experiment, only the ephippia containing resting eggs were used. Under the optimum environmental parameters (temperature 25±2°C, pH 5-9, photoperiod ≥8 hours light per day) none of the ephippia containing resting eggs hatched. These "weak eggs" might be caused by the maternal factor where the production ephippia is not normally occurs. The mechanism for hatching process is not well developed yet in this clone of *M. micrura.* In the earlier experiments, *M. micrura* reproduced by both asexual and sexual reproduction. Hence, genetic variation within asexual and sexual generation of *M. micrura* was investigated. Gene sequences of 16S rRNA mtDNA and Cytochrome C Oxidase Subunit 1 were used. Both genes show 100% similarity of the nucleotide sequences between different generations of asexual *M. micrura.* However, comparison of the 16S rRNA mtDNA gene sequences between asexual and sexual generations shows that the level of

similarity between the sequences was only 55.80 to 56.82%, whereas Cytochrome C Oxidase Subunit 1 gene sequences shows 49.50 to 58.86% of similarity. This study clearly illustrated that production of ephippia in tropical *M. micrura* requires combination of low food availability and crowding condition. The failure of the eggs to hatch could be due to low synchronization of male production and ephippia formation in tropical *M. micrura*. The study also showed that sexual reproduction produces a new generation of *M. micrura* with higher genetic variation.

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

PENGHASILAN EPIPIA DAN BIODIVERSITI GENETIK DALAM KLADOCERA TROPIKA *Moina micrura* Kurz

Oleh

MOHD AZURAIDI BIN OSMAN

Jun 2012

Pengerusi: Profesor Mariana Nor Shamsudin, PhD

Institut : Institut Biosains

Di Malaysia, naupli *Artemia* yang diimport merupakan diet standard yang digunakan dalam industri akuakultur kerana ianya boleh didapati dalam bentuk dorman. Walau bagaimanapun, makanan hidup yang diimport ini adalah mahal dan hanya boleh hidup beberapa jam di dalam air tawar. Oleh itu, makanan hidup tempatan yang senang didapati dan murah yang boleh menjadi alternatif kepada spesis import ini adalah diperlukan. Dalam kajian ini, kesan perbezaan kepekatan makanan dan kekerapan memberi makan terhadap penghasilan epipia telah disiasat. Beberapa kepekatan makanan berbeza yang mengandungi *Nannochloropsis oculata* ($4x10^6$ sel/ml, $4x10^4$ sel/ml, $4x10^2$ sel/ml dan tanpa makanan sebagai kawalan) dan kekerapan memberi makan (3 hari, 3 dan 5 hari, 5 hari, 8 hari, 8 dan 3 hari dan setiap hari) telah digunakan. Bilangan purata epipia tertinggi (7.33±1.53 epipia/l) dihasilkan apabila kultura diberi makan setiap 3 dan 5 hari secara berselang seli. Apabila kultura diberi makan dengan $4x10^6$ sel/ml, tiada epipia

dihasilkan kerana terdapat kepekatan makanan yang tinggi. Dalam eksperimen yang berikutnya, pembiakan partinogenesis, kehadiran jantan dan penghasilan epipia dalam *M. micrura* juga dikaji. Dua ratus individu betina berumur kurang daripada 24 jam digunakan. Kultura diberi makan dengan *N. oculata* pada kepekatan berbeza ($4x10^6$ sel/ml, $4x10^4$ sel/ml, $4x10^2$ sel/ml dan tanpa makanan sebagai kawalan) untuk tempoh 12 hari. Jumlah jantan yang paling banyak (186.7±23.1 jantan/l) telah dihasilkan oleh kultura yang diberi makan dengan $4x10^2$ sel/ml *N. oculata* apabila kepadatan populasi mencecah > 1600 individu/l. Sama juga, purata bilangan epipia terbanyak (160.0±0.0 epipia/l) telah dihasilkan oleh *M. micrura* yang telah diberi makan dengan $4x10^2$ sel/ml *N. oculata* apabila kepadatan populasi mencecah > 2000 individu/l. Kombinasi kedua-dua faktor iaitu bekalan makanan yang terhad dan kepadatan populasi yang tinggi diperlukan untuk merangsang penghasilan epipia. Kebolehan menetas epipia yang dihasilkan daripada eksperimen terdahulu juga telah dikaji. Dalam eksperimen ini, hanya epipia yang mengandungi telur dorman digunakan. Di bawah keadaaan persekitaran yang optimum (suhu 25±2°C, pH 5-9, fotokala ≥8 jam cahaya sehari) tiada satu pun epipia berjaya menetas. "Telur yang lemah" ini mungkin disebabkan oleh faktor induk dimana penghasilan epipia biasanya tidak berlaku. Jadi, mekanisma proses penetasan belum cekap bagi klon *M. micrura* ini. Dalam eksperimen terawal, *M. micrura* menjalani pembiakan secara aseksual dan seksual. Maka, variasi genetik diantara generasi aseksual dan seksual *M. micrura* juga telah dikaji. Jujukan gen 16S rRNA mtDNA dan Cytochrome C Oxidase Subunit 1 telah digunakan. Kedua-dua jujukan gen ini menunjukkan 100% persamaan jujukan nukleotida antara

beberapa generasi aseksual *M. micrura.* Namun, perbandingan antara jujukan gen 16S rRNA mtDNA antara generasi aseksual dan generasi seksual menunjukkan tahap persamaan jujukan gen hanyalah diantara 55.80 – 56.82%, manakala jujukan gen Cytochrome C Oxidase Subunit 1menunjukkan 49.50 – 58.86% persamaan. Kajian ini jelas menunjukkan penghasilan epipia dalam *M. micrura* tropika memerlukan kombinasi faktor makanan terhad dan populasi yang padat. Kegagalan telur dorman untuk menetas berkemungkinan disebabkan oleh penyelarasan penghasilan jantan dan telur dorman yang rendah. Kajian ini juga menunjukkan pembiakan seks menghasilkan generasi baru *M. micrura* dengan variasi genetik yang lebih tinggi.

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

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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 other institutions.

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TABLE OF CONTENTS