UNIVERSITI PUTRA MALAYSIA

MOLECULAR PHYLOGENETICS OF SELECTED *CLARIAS* SPP. INFERRED FROM MITOCHONDRIAL DNA AND NUCLEAR GENES

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master 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

MOLECULAR PHYLOGENETICS OF SELECTED *CLARIAS* SPP. INFERRED FROM MITOCHONDRIAL DNA AND NUCLEAR GENES

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Chair: Siti Khalijah Daud, PhD

Faculty: Faculty of Science

Clarias spp., belonging to the family Clariidae, are among the important fishes in aquaculture and fisheries in Malaysia. A total of 37 samples were collected from five different geographic locations in Malaysia, namely Pahang, Kelantan, Perak, Selangor and Sarawak. In the present study, the phylogenetic levels of four species of catfish were assessed using sequences of the cytochrome b (Cyb b), cytochrome oxidase subunit 1 (CO1) and a large subunit of 16s rRNA (16s rRNA) mitochondrial DNA, as well as a nuclear gene, the Recombination Activating Genes 2 (RAG2). The methods used to infer the phylogeny of this genus were neighbour joining, maximum parsimony, maximum likelihood and Bayesian analyses using MEGA4, PAUP and Mr. Bayes softwares. Thirty seven sequences of *C. batrachus, C. macrocephlaus, C. nieuhofii* and *C. gariepinus* were obtained and they were aligned together with outgroups of *Pangasianodon gigas* and *Ictalurus punctatus* from GeneBank. The results showed a monophyletic group of Asian *Clarias* and they are different from African catfish, *C. gariepinus*. These results were supported with significant bootstrap values. Approximately, 552bp were sequenced from

73 individuals of *C. batrachus.* There were high levels of within population but low level of interpopulation variabilities among the five populations of *C. batrachus* in Malaysia. Haplotype diversity and nucleotide diversity ranged from 0.0000 to 0.8333 and from 0.0000 to 0.0112, respectively. Twelve haplotypes were detected amongst the five *C. batrachus* populations. The results indicated that the haplotype diversity observed in Kelantan was the highest (0.8333), while the Sarawak population had the lowest (0.0000). Based on NJ and ML phylogenetic trees, the five populations of *C. batrachus* were divided into two clades, namely Clade I and Clade II. Clade I was further divided into subclades **a** and **b**. Hap1 and Hap2 are the common haplotypes found in three populations (Perak, Pahang and Johor) and these haplotypes were considered as the ancestral haplotypes for Clade I. However, Hap7 (Sarawak population) was also clustered together in Clade I. This may be due to the gene flow that occurred among islands of Sumatera, Borneo and Southeast Asia during the exposed Sunda Shelf in the Pleistocene Epoch. Clade II consisted of Hap8 to Hap12 which belonged to the Kelantan population. All populations of *C. batrachus* in this study were monophyletic to each other with Kelantan population being the most diverse. The low level of haplotype diversity in Sarawak population could be due to small samples used (n=4), past bottleneck events or the presence of physical barriers for gene flow among the populations, thus Sarawak populations have their own unique haplotypes. The genetic structure of *C. batrachus* agrees with previous study whereby the changes in sea level during the Pleistocene Epoch had little influence in shaping the biodiversity of Sunda Shelf. In order to validate the monophyletic status of Asian *Clarias,* more species and samples should be included in future studies.

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

KAJIAN MOLEKUL FILOGENI SPESIES *CLARIAS* TERPILIH BERDASARKAN DNA MITOKONDRIA DAN GEN NUKLEAR

Oleh

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Clarias spp., tergolong dalam famili Clariidae, adalah di antara ikan yang penting dalam akuakultur dan perikanan di Malaysia. Sebanyak 37 sampel telah dikumpulkan daripada lima lokasi geografi yang berlainan di Malaysia, iaitu Pahang, Kelantan, Perak, Selangor dan Sarawak. Dalam kajian ini, kami menilai aras filogeni empat spesies ikan keli dengan menggunakan jujukan DNA mitokondria sitokrom b (Cyt b), sitokrom oksida subunit I (COI) dan subunit besar 16s rRNA (16s rRNA), serta satu gen nuklear iaitu Gen Aktifan Rekombinan 2 (RAG2). Kaedah yang digunakan untuk membina pokok filogeni ialah 'neighbour joining', 'maximum parsimony', 'maximum likelihood' dan 'Analisis Bayesian' dengan menggunakan perisian MEGA4, PAUP dan Mr. Bayes. Sejumlah 37 jujukan *C. batrachus, C. macrocephalus, C. nieuhofii* dan *C. gariepinus* telah diperolehi dan semuanya dijajarkan bersama dengan dua spesies 'outgroup' iaitu *Pangasianodon gigas* dan *Ictalurus punctatus* dari GeneBank. Keputusan menunjukkan ikan keli Asia adalah monofiletik yang mana ia berbeza dengan keli Afrika, *C. gariepinus* Keputusan ini disokong dengan nilai 'bootstrap' yang signifikan. Dianggarkan 552bp telah dijujukkan daripada 73 individu *C. batrachus.* Terdapat nilai variabiliti yang tinggi di dalam

populasi tetapi nilai variabiliti yang rendah antara populasi bagi kelima-lima populasi *C. batrachus* di Malaysia. Diversiti haplotip dan nukleotid masing-masing berjulat dari 0.0000 hingga 0.8333 dan dari 0.00000 hingga 0.001121. Dua belas haplotip telah dikesan daripada lima populasi *C. batrachus* tersebut. Keputusan menunjukkan bahawa diversiti haplotip bagi populasi Kelantan adalah tertinggi (0.8333) manakala populasi Sarawak pula adalah terendah (0.0000). Berdasarkan kepada pokok filogeni NJ dan MP, lima populasi *C. batrachus* dibahagikan kepada dua klad, iaitu Klad I dan Klad II. Klad II dibahagikan lagi kepada subklad **a** dan **b**. Hap1 dan Hap2 adalah haplotip yang biasa dijumpai dalam tiga populasi (Perak, Pahang dan Johor) malah ia juga di anggap halotip leluhur bagi Klad I. Hap7 (populasi Sarawak) dikelompokkan bersama dengan Klad I kerana kemungkinan berlakunya aliran gen antara kepulauan Sumatera, Borneo dan Asia Tenggara semasa penonjolan pentas Sunda pada zaman Pleistocene. Klad II pula terdiri daripada Hap8 hingga Hap12 yang dipunyai oleh populasi Kelantan. Kesemua populasi *C. batrachus* dalam kajian ini adalah monofiletik antara satu sama lain dengan populasi Kelantan adalah yang paling pelbagai. Paras diversiti yang rendah pada populasi Sarawak mungkin disebabkan oleh saiz sampel yang kecil (n=4), kejadian ‘leher botol’ pada masa lampau atau wujudnya halangan fizikal bagi aliran gen antara populasi, oleh itu populasi Sarawak mempunyai haplotip gen sendiri yang unik. Struktur genetik *C. batrachus* sama dengan kajian lepas di mana perubahan aras laut semasa zaman Pleistocene hanya menyebabkan sedikit sahaja perubahan kepada biodiversiti di Pentas Sunda. Bagi mengesahkan status monofiletik bagi *Clarias* Asia, lebih banyak spesies dan sampel perlu digunakan bagi kajian masa hadapan.

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I certify that the an Examination Committee has met on date of viva voce to conduct the final examination of Nor Hasnita binti Othman on her Master of Science thesis entitled "Molecular Phylogenetics of selected *Clarias* spp. inferred from mitochondrial DNA and nuclear genes" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student to be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been eccepted 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 dully 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.

NOR HASNITA BINTI OTHMAN

Date: 8 May 2012

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