UNIVERSITI PUTRA MALAYSIA

MOLECULAR SYSTEMATICS OF MALAYSIAN MAHSEER AND PHYLOGEOGRAPHY OF *Tor douronensis* (VALENCIENNES)

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MOLECULAR SYSTEMATICS OF MALAYSIAN MAHSEER AND PHYLOGEOGRAPHY OF *Tor douronensis* (VALENCIENNES)

By

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Faculty : Science

Mahseer or commonly known as ‘ikan Kelah’ is an indigenous species of Malaysia. The popularity of this freshwater fish among the locals has made it an economical important aquaculture candidate. This study examines the molecular systematics among Malaysian Mahseers namely *Tor douronensis* (N=7), *Tor tambroides* (N=4) and *Neolissochilus stracheyi* (N=7) and their relationships with other Asian cyprinids *(Ctenopharynodon idella, Aristichthys nobilis, Mylopharyngodon piceus, Hypophthalmichthys molitrix, Opsariichthys bidens, Carrasius auratus, Barbonymus gonionotus and Danio rerio)* using direct sequencing of mitochondrial DNA genes (cytochrome oxidase subunit II, 12S ribosomal RNA and ATPase6/8) and a nuclear gene (beta-actin). The phylogenetic trees were constructed using Neighbour Joining, Maximum Parsimony and Bayesian analyses.

This study also describes the DNA mitochondrial diversity and genetic structure of *T. douronensis* (N=134) in Sarawak river systems based on cytochrome *c* oxidase I gene

sequences. The nucleotide diversity index was used to estimate within population genetic diversity. While, DNA sequence polymorphism program was used to generate haplotype sequences and later the genetic relationships among haplotypes were determined using the reduced median network and by constructing phylogenetic trees through Neighbour Joining and Maximum Parsimony methods. The test of genetic structuring of *T. douronensis* in Sarawak was employed using analysis of molecular variance (AMOVA) implemented using ARLEQUIN program.

The results of phylogenetic study from these two gene mitochondrial DNA and a nuclear gene strongly support the reciprocally monophyletic status between genus *Tor* (*T. douronensis* and *T. tambroides*) and genus *Neolissochilus* (represented by *N. stracheyi*). Thus, the findings have strengthened the current taxonomic status for all three Malaysian indigenous Mahseer. For the Asian cyprinids, an interesting finding was that *A. nobilis* and *H. molitrix* were phylogenetically similar and was clustered consistently in the same subclade and was supported with a very low Kimura-2-Parameter distance value (0.000).

The mitochondrial DNA diversity and population structure analyses inferred from cytochrome *c* oxidase I sequences found low level of intra and high level of inter population variations in samples of *T. douronensis* of Sarawak. The presence of unique haplotype in some populations, along with high F_{ST} values indicated that there has been restricted or no migration among the existing populations which were separated by geographical barrier or river systems. The constructed Neighbour Joining (NJ) and Maximum Parsimony (MP) trees based on haplotypes showed similar topologies, in which the southern Sarawak population was grouped in Cluster

I while northern and central Sarawak were clustered in Cluster II. Population structure of the three regions was analysed using Analysis of Molecular Variance (AMOVA). Most of the variations occurred among regions and among subpopulations within a region. The p value after Bonferroni correction revealed that the pairwise F_{ST} values for some populations of southern Sarawak (Bau – Terbat, Terbat – Semadang, Bau – Semadang) and population of northern Sarawak (Kg. Pa Puti - Tuyo River) were not significantly different (P>0.05). The sharing of haplotype HapTD1 between samples from Semadang (91.3%), Bakun Dam (25%), Bau (86.4%), Entabai River (6.7%) and Terbat (100%) indicated the historical interconnection of drainages of southern and central Sarawak during the Tertiary and Quaternary Periods via the Sunda River (10-5 mya).

Overall, the phylogenetic analyses of the three mitochondrial genes and a nuclear gene have provided better insights into the evolutionary relationships and taxonomy analysis of the Asian cyprinids in relation to Malaysian Mahseer. The phylogenetic results generated from both types of genes were generally in agreement and concordant with the current classification of the cyprinids based on morphological and meristic characters. The population structure analysis using cytochrome *c* oxidase I gene sequences has shown high heterogeneity among most of *T. douronensis* populations in Sarawak which could possibly because of low level of gene flow between the populations. These findings have important implications for aquaculture and fisheries management of this indigenous species of Malaysian Mahseers.

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

SISTEMATIK MOLEKUL IKAN KELAH MALAYSIA DAN FILOGEOGRAFI *Tor douronensis* (VALENCIENNES)

Oleh

NADIATUL HAFIZA HASSAN

Februari 2012

Pengerusi : Professor Madya Siti Khalijah Daud, PhD

Fakulti : Sains

Mahseer atau lebih dikenali sebagai ikan kelah adalah ikan tempatan di Malaysia. Populariti ikan air tawar ini di kalangan penduduk tempatan menjadikannya calon spesies akuakultur yang penting dari sudut ekonomi. Kajian ini dijalankan untuk melihat sistematik molekul ikan kelah di Malaysia iaitu *Tor douronensis* (N=7), *Tor tambroides* (N=4) dan *Neolissochilus stracheyi* (N=7) serta hubungannya dengan ikan siprinid Asia yang lain (*Ctenopharynodon idella, Aristichthys nobilis, Mylopharyngodon piceus, Hypophthalmichthys molitrix, Opsariichthys bidens, Carrasius auratus, Barbonymus gonionotus and Danio rerio*) dengan menggunakan analisis penjujukan terus gen daripada DNA mitokondria (sitokrom oksida subunit II, 12S ribosomal RNA dan ATPase 6/8) dan gen nuklear (beta-aktin). Pohon filogeni di bina menggunakan *Neighbour Joining*, Parsimoni Maksimum dan analisis Bayesian.

Kajian ini juga menghuraikan tentang kepelbagaian struktur genetik bagi *T. douronensis* (N=134) dalam sistem sungai di Sarawak berdasarkan kepada jujukan-

jujukan gen sitokrom *c* oksida I. Indeks kepelbagaian nukleotid digunakan untuk menganggar kepelbagaian genetik di dalam populasi. Manakala, program polimorfisme jujukan DNA digunakan untuk menghasilkan jujukan haplotip dan kemudiannya perhubungan antara haplotip ditentukan dengan menggunakan pengurangan jaringan tengah dan dengan membina pohon filogeni menggunakan kaedah *Neighbour Joining* dan Parsimoni Maksimum. Kajian bagi penstrukturan genetik *T. douronensis* di Sarawak dijalankan dengan menggunakan analisis varians molekul (AMOVA) yang diimplementasikan menggunakan program ARLEQUIN.

Keputusan kajian filogenetik daripada dua gen DNA mitokondria dan gen nuklear menyokong kuat status monofiletik timbal balik antara genus *Tor* di Malaysia (*T. douronensis* dan *T. tambroides*) dan genus *Neolisochilus* (yang diwakili oleh *N. stracheyi*). Oleh itu, keputusan ini menguatkan lagi status taksonomi terkini untuk ketiga-tiga ikan kelah tempatan di Malaysia. Bagi siprinid di Asia, penemuan menarik ialah *A. nobilis* dan *H. molitrix* adalah serupa dari segi filogenetik dan dikelompokkan dalam subklad yang sama pada nilai jarak Parameter-2-Kimura yang sangat rendah (0.000).

Kepelbagaian DNA mitokondria dan struktur populasi yang dianggarkan daripada jujukan sitokrom *c* oksida I mendapati variasi yang rendah bagi intrapopulasi dan variasi yang tinggi di antara populasi sampel *T. douronensis* di Sarawak. Kehadiran haplotip unik di beberapa populasi, disertai dengan nilai F_{ST} yang tinggi menunjukkan terdapat kekangan atau ketiadaan migrasi yang berlaku antara populasi yang dipisahkan oleh sempadan geografi ataupun sistem sungai. Pohon *Neighbour Joining* dan Parsimoni Maksimum yang dibina berasaskan haplotip menunjukkan topologi yang sama, di mana populasi selatan Sarawak di kelompokkan dalam Kluster I manakala utara dan tengah Sarawak pula diletakkan dalam Kluster II. Struktur populasi bagi tiga kawasan tersebut dianalisis dengan menggunakan AMOVA. Hasil keputusan mendapati kebanyakan variasi berlaku antara kawasan dan antara populasi dalam satu kawasan. Nilai p selepas pembetulan Bonferonni menunjukkan perbezaan nilai pasangan F_{ST} yang tidak signifikan ($p>0.05$) yang melibatkan sesetengah populasi di selatan Sarawak (Bau-Terbat, Terbat-Semadang, Bau-Semadang) dan populasi di utara Sarawak (Kg. Pa Puti-Sg. Tuyo). Perkongsian haplotip HapTD1 antara sampel dari Semadang (91.3%), Bakun Dam (25.0%), Bau (86.4%) dan Sg. Entabai (6.7%) mendedahkan bahawa, terdapat sejarah perhubungan di antara sungai-sungai di selatan dan tengah Sarawak melalui Sungai Sunda semasa zaman Tertier dan Kuatenari (10-5 ribu tahun yang lalu).

Secara keseluruhannya, analisis filogenetik bagi dua gen mitokondria dan gen nuklear mampu memberikan satu pandangan yang lebih baik tentang hubungan evolusi dan analisis taksonomi siprinid di Asia dengan ikan kelah. Penemuan filogenetik yang dihasilkan daripada dua jenis gen adalah selari dengan pengkelasan terkini siprinid berdasarkan kepada ciri morfologi dan meristik. Analisis struktur populasi menggunakan jujukan gen sitokrom *c* oksida I menunjukkan heterogeniti yang tinggi bagi kebanyakan populasi *T. douronensis* di Sarawak yang berkemungkinan disebabkan oleh aliran gen yang terhad antara populasi. Hasil kajian ini mempunyai implikasi yang penting untuk akuakultur dan pengurusan perikanan spesies ikah kelah tempatan di Malaysia.

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I certify that a Thesis Examination Committee has met on 23rd February 2012 to conduct the final examination of Nadiatul Hafiza binti Hassan on her thesis entitled "Molecular Systematics of Malaysian Mahseers and Phylogeography of *Tor douronensis* (Valenciennes)" 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.

Members of the Thesis Examination Committee were as follows:

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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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Date:

DECLARATION

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

NADIATUL HAFIZA BINTI HASSAN

Date: 23 February 2012

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