MORPHOLOGICAL AND GENETIC IDENTIFICATION, AND POPULATION STRUCTURE OF KELAH (*TOR TAMBROIDES*)

By

REMMY KEONG BUN POH

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Chairman: **Associate Professor Siti Shapor Siraj, PhD**

Faculty: **Science**

Tor tambroides, locally known as kelah, is one of the most sought after of the freshwater fishes in the country, both as food as well as a highly priced game fish. The identification of this fish into various groups and names is largely based on external features and colours. Thus, this study was undertaken to determine morphological differences, genetic variation and genetic relationships among three populations of *Tor tambroides.* Samples of kelah were taken from three locations, namely Pahang, Negeri Sembilan and Kelantan. A total of sixty three samples were used with weights ranging from 3.1 to 1800 g, total lengths ranging from 5.5 to 50.7 cm and standard lengths ranging from 4.2 to 40.0 cm.

Morphological studies based on conventional and truss morphometric and meristic methods were applied on each sample of fish. The non-quantitative morphology of kelah from the three populations did not show any distinct differences. However, the presence of a median lobe and large scales with dark vertical bands especially on the scales above the lateral line are the most distinctive features of kelah that can differentiate this fish based on phenotypic observations. The range of gill rakers and vertebrae numbers were 17 to 23 and 30 to 34, respectively. The number of branchiostegals rays was three. The meristic characters showed greater differences among the *Tor tambroides* populations than the morphometric characters. The mean number of caudal fin rays, upper lateral line scales, lower lateral line scales and lateral line scales showed significant differences ($P<0.05$) among populations. The four meristic characters summarized by the canonical discriminant function analysis plots together with both the hierarchical clustering analyses based on five conventional morphometric and four meristic characters, respectively showed similarities in shape, fin or scale counts of kelah from the three populations.

For Randomly Amplified Polymorphic DNA (RAPD) markers, a total of 20 short arbitrary primers were screened but only twelve primers were used. The values of the genetic distances generated using 226 RAPD markers ranged from 0.7548 to 0.7643. In addition, thirty eight microsatellite markers developed for *Mystus nemurus* were used in this study. Only five primer pairs which amplified bands at the expected allele size regions were used for characterizing this species. The observed heterozygosity values were higher than the expected heterozygosity values for the bands amplified by primer pairs MnSC4-3B, MnLR2-1-52A and MnRmC3-1 across the three populations but the bands amplified by primer pairs MnSC4-1A and MnLR2-1-17B showed lower observed heterozygosity values than the expected heterozygosity values. The mean F_{IS} value across the three populations was negative, indicating no deficit in heterozygosity. The mean value of F_{ST} or the fixation index was low indicating no gene was fixed within populations relative to the total population and the populations were still high in

gene variation. The high value of N_m suggested high gene flow among the three populations. Both the (χ^2) chi-square and the (G^2) likelihood ratio tests significant differences ($P<0.05$), indicating deviations from Hardy-Weinberg equilibrium in most loci except for one locus (MnSC4-1A) in the Negeri Sembilan population and two loci (MnLR2-1-52A and MnSC4-1A) in the Kelantan population. The genetic distance values generated by the microsatellite markers ranged from 0.1053 to 0.1960. The UPGMA dendrograms constructed from the genetic distances based on both the RAPD and the microsatellite markers showed similar clustering patterns in that the Negeri Sembilan and Kelantan populations shared a similar cluster while the Pahang population was away by itself.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
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PENGENALPASTIAN SECARA MORFOLOGI DAN GENETIK, SERTA STRUKTUR POPULASI BAGI KELAH (*TOR TAMBROIDES*)

Oleh

REMMY KEONG BUN POH

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Pengerusi: Profesor Madya Siti Shapor Siraj, PhD

Fakulti: Sains

Tor tambroides, dikenali dengan nama tempatan sebagai kelah merupakan salah satu daripada ikan air tawar yang digemari di negara ini, sebagai sumber makanan dan ikan pancing yang bernilai tinggi. Identifikasi ikan ini kepada pelbagai kumpulan dan nama kebanyakannya berdasarkan kepada sifat luaran dan warna. Oleh itu, kajian ini dijalankan untuk menentukan perbezaan morfologi, variasi genetik dan hubungan genetik antara tiga populasi *Tor tambroides.* Sampel kelah diperolehi dari tiga lokasi iaitu Pahang, Negeri Sembilan dan Kelantan. Sejumlah enam puluh tiga sampel telah digunakan dengan julat berat dari 3.1 ke 1800 g, julat panjang penuh dari 5.5 ke 50.7 cm dan julat panjang piawai dari 4.2 to 40.0 cm.

Kajian morfologi yang berasaskan kepada kaedah morfometrik konvensional dan truss serta meristik telah dijalankan bagi setiap sampel ikan. Morfologi kelah secara bukan kuantitatif daripada tiga populasi tidak menunjukkan perbezaan yang ketara. Namun, kehadiran lobus median dan sisik besar yang dikelilingi jalur gelap terutama sisik di atas garisan lateral merupakan ciri-ciri ketara yang boleh

membezakan ikan ini berdasarkan kepada pemerhatian fenotip. Julat bilangan sisir insang dan tulang vertebra masing-masing adalah dari 17 hingga 23 dan 30 hingga 34. Jumlah bilangan ruji ‘branchiostegal’ adalah tiga. Ciri meristik menunjukkan perbezaan yang lebih besar antara populasi *Tor tambroides* berbanding dengan ciri morfometrik. Min bilangan ruji sirip kaudal, sisik bahagian atas garis lateral, sisik bahagian bawah garis lateral dan sisik garis lateral menunjukkan perbezaan bererti ($P<0.05$) di antara populasi. Empat ciri meristik (m) disimpulkan oleh plot ‘canonical discriminant function analysis’ berserta dengan kedua-dua analisis pengelompok hierarki yang berdasarkan lima ciri morfometrik dan empat ciri meristik masing-masing menunjukkan kesamaan dari segi bentuk, bilangan sirip atau sisik bagi kelah pada ketiga-tiga populasi.

Bagi kajian “Randomly Amplified Polymorphic DNA (RAPD)’, sejumlah 20 primer arbitrari pendek telah diskrin dan hanya 12 lokus telah digunakan. Nilai jarak genetik yang terhasil dengan menggunakan 226 penanda RAPD adalah berjulat dari 0.7548 hingga 0.7643. Tambahan pula, tiga puluh lapan lokus penanda mikrosatelit yang telah dibangunkan untuk *Mystus nemurus* telah digunakan dalam kajian ini. Hanya lima primer yang menghasilkan alel pada jarak alel terjangka telah digunakan bagi pencirian species ini. Nilai paras heterozigot terhasil adalah lebih tinggi daripada nilai paras heterozigot terjangka untuk alel yang terhasil oleh penanda mikrosatelit MnSC4-3B, MnLR2-1-52A and MnRmC3-1 untuk ketiga-tiga populasi tetapi alel yang terhasil oleh penanda mikrosatelit MnSC4-1A and MnLR2-1-17B menunjukkan nilai paras heterozigot terhasil yang lebih rendah daripada paras heterozigot terjangka. Nilai min F_{IS} untuk ketiga-tiga populasi adalah negatif menunjukkan tiada pengurangan dalam

keheterozigosan. Nilai F_{ST} atau ‘fixation index’ yang rendah menunjukkan tiada gen yang tetap antara populasi dinisbahkan dengan jumlah populasi. Nilai N_m yang tinggi mencadangkan pemindahan gen yang tinggi antara ketiga-tiga populasi. Kedua-dua ujian ‘chi-square’ (χ^2) dan ujian ‘likelihood ratio’ (G^2) menunjukkan perbezaan bererti ($P<0.05$), bermaksud persimpangan daripada keseimbangan ‘Hardy-Weinberg’ untuk hampir kesemua lokus kecuali satu lokus (MnSC4-1A) dalam populasi Negeri Sembilan dan dua lokus (MnLR2-1-52A dan MnSC4-1A) dalam populasi Kelantan. Nilai jarak genetik yang terhasil daripada penunjuk mikrosatelit adalah rendah berjulat dari 0.1053 hingga 0.1960. UPGMA dendrogram yang dibina daripada nilai jarak genetik bagi kedua-dua penanda RAPD dan mikrosatelit menunjukkan corak pengelompokan yang sama iaitu populasi Negeri Sembilan dan Kelantan berada dalam satu kumpulan yang sama manakala populasi Pahang terasing secara bersendirian.

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I certify that an Examination Committee has met on 5th June 2006 to conduct the final examination of Remmy Keong Bun Poh on his Master of Science thesis entitled "Morphological and Genetic Identification, and Population Structure of Kelah (*Tor tambroides*)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Aziz Arshad, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Tan Soon Guan, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal examiner)

Jothi M. Panandam, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal examiner)

Siti Azizah Mohd Nor, PhD
Associate Professor
School of Biological Sciences
Universiti Sains Malaysia
(External examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Siti Shapor Siraj, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Siti Khalijah Daud, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

REMMY KEONG BUN POH

Date: 10 July 2006

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