



UNIVERSITI PUTRA MALAYSIA

**MORPHOMETRIC ANALYSIS AND POPULATION GENETICS OF
MAHISEFID (*Rutilus frisii kutum* KAMENSKY) OF THE SOUTH
CASPIAN SEA**

HOSSEIN ALI ABDOLHAY

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**MORPHOMETRIC ANALYSIS AND POPULATION GENETICS OF
MAHISEFID (*Rutilus frisii kutum* KAMENSKY) OF
THE SOUTH CASPIAN SEA**

By

HOSSEIN ALI ABDOLHAY

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Chairperson: Assoc. Professor Siti Khalijah Daud, PhD

Faculty: Science

Mahisefid is one of the most important and economical fishes in the southern area of the Caspian Sea. The distribution of Mahisefids is from Kura in Azerbaijan to Turkmenistan Republic. Mahisefids migrate to rivers for spawning. The present study aimed to determine morphometric characters, population structure and genetic diversity of Mahisefids. Mahisefid populations were studied from Lamir Rivers and Sefid Rud Rivers in the Guilan Province, as well as Shir Rud Rivers and Tajan Rivers in the Mazandaran Province. A total 387 Mahisefid samples (male and female) were collected in Spring 2005 from four different rivers, where they migrate for their annual migrations. Fourteen conventional morphometrics, 13 ratios morphometric, twelve Truss morphometrics and the ratios to fork length were selected for this study. Conventional and Truss morphometric data of *R. frisii kutum* from these four rivers were analyzed using two-way ANOVA, discriminant and Principal Component



Analysis (PCA). The results showed that all morphometric characters in males and females were significantly different ($P < 0.05$) among the four rivers. Based on conventional morphometric characters there, was no clustering of Mahisfid populations from the four rivers for both male and female populations,; i.e. However, on the basis of Truss morphometric characters, the pattern of clustering was slightly different in which there was no clustering for male but there was two clusters for female populations. Population of Sefid Rud River, Shir Rud River and Tajan River populations in one cluster while Lamir river population in another cluster.

For RFLP, 294 samples were used and the PCR products were digested by 20 restriction enzymes, namely *TasI*, *HaeIII*, *HinfI*, *HincII*, *Sall*, *DraI*, *AccI*, *AvaII*, *XhaI*, *BshNI*, *AvaI*, *BclI*, *BshII*, *MspI*, *PstI*, *RsaI*, *SdnI*, *TaqI*, *TruI*, and *VspI*. The four restriction enzymes namely *TasI*, *HaeIII*, *HinfI*, *HincII* showed polymorphism, six enzymes did not have any restriction, and 14 enzymes showed monomorphic bands. A total 20 of haplotypes were observed, in which AAAA and BAAA had the highest frequency. The average haplotype frequency of AAAA was 29.93% and the average haplotype frequency of BAAA was 27.55%. Haplotype BDAB was unique for the Sefid Rud River populations, haplotypes ADAA and ABAC were unique for the Lamir River population, haplotype BAAB was unique for the Shir Rud River population while haplotype of BAAC was unique for the Tajan River population. Based on RFLP marker, the four populations of Mahisfid were divided into two main clusters, i.e. Shir Rud River and Lamir River populations in one cluster while Tajan River and Sefid Rud River in another cluster. This clustering was not in accordance to the geographical distance of four rivers populations.



In microsatellites analysis, 120 specimens (30 samples from each river) of *R. frissikutum* from four rivers were caught. Thirty microsatellite primers were used of which eight primers showed polymorphism. Heterozygosity over all samples was observed among loci that ranged from 0.13 to 0.91. For a given locus, the observed heterozygosity varied greatly among the samples. The highest H_e was 0.92 at locus SYP4 in Shir Rud River and the lowest H_e was 0.24 at locus SYP6 in Tajan River. The F_{st} values between the four rivers were significantly different ($P < 0.05$) suggesting that all of the four populations were significantly differentiated that can be explained by their geographical distances. There were significant deviations ($P < 0.05$) from Hardy-Weinberg Equilibrium at all loci and all rivers except at locus SPY6. The dendrogram showed that there were two major clusters in Mahisefid population i.e. The Shir Rud population was in one cluster, while the remaining three populations were in another cluster with a genetic distance value of 0.064.

The results of morphometrics, RFLP and microsatellite markers from this study are important to be taken into account by policy makers and fishery managers for stock enhancement program of Mahisefid in Iran. This study also suggested that Mahisefid populations from different river systems should be treated separately since they have different and should not be mixed especially for induce breeding activities.

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

**ANALISIS MORFOMETRIK DAN POPULASI GENETIK MAHISEFID
(*Rutilus frisii kutum* KAMENSKY) DI SELATAN LAUT CASPIAN**

Oleh

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Mahisefid adalah salah satu spesies ikan yang penting dari segi ekonomi di selatan kawasan Laut Caspian. Taburan Mahisefid adalah dari Kura di Azerbaijan hingga ke Republik Turkmenistan. Mahisefid berhijrah ke sungai untuk bertelur. Kajian ini bertujuan untuk menentukan ciri morfometrik, struktur populasi dan kepelbagaian genetik Mahisefid. Populasi Mahisefid dari Sungai Lamir dan Sungai Sefid Rud di daerah Guilan, serta populasi Sungai Shir Rudd dan Sungai Tajan di daerah Mazandaran digunakan dalam kajian ini. Sejumlah 387 sampel (jantan dan betina) Mahisefid telah ditangkap pada musim bunga 2005 dari empat sungai yang berbeza di mana ikan ini setiap tahun berhijrah untuk bertelur. Empat belas ciri morfometrik konvensional, 13 nisbah dan 12 morfometrik Truss dipilih untuk kajian ini. Data morfometrik konvensional dan Truss bagi *R. frisii kutum* dari empat sungai ini dianalisis menggunakan ANOVA dua-hala, diskriminan dan Analisis Komponen Prinsip. Hasil kajian menunjukkan semua ciri morfometrik pada jantan dan betina mempunyai perbezaan bererti ($P < 0.05$) di kalangan empat sungai tersebut. Berdasarkan

kepada ciri morfometrik konvensional, tiada kelompok bagi populasi jantan dan betina Mahisefid dari empat sungai yang dikaji. Walau bagaimanapun, berdasarkan ciri morfometrik Truss, pola kelompok adalah sedikit berbeza di mana tidak terdapat kelompok bagi jantan tetapi terdapat dua kelompok bagi populasi betina, i.e. Populasi Sungai Sefid Rud, Sungai Shir Rud dan Sungai Tajan dalam satu kumpulan dan populasi Sungai Lamir dalam kumpulan satu lagi.

Untuk RFLP, 294 sampel telah digunakan dan produk PCR dihadamkan dengan menggunakan 20 enzim pembatas, iaitu *TasI*, *HaeIII*, *HinfI*, *HincII*, *SalI*, *DraI*, *AccI*, *AvaII*, *XhaI*, *BshNI*, *AvaI*, *BclI*, *BshII*, *MspI*, *PstI*, *RsaI*, *SdnI*, *TaqI*, *TruI*, *VspI*. Daripada 20 enzim yang dikaji, empat enzim pembatas iaitu *TasI*, *HaeIII*, *HinfI* dan *HincII* adalah polimorfik, 6 enzim tiada penghad dan 14 enzim lagi adalah monomorfik. Sejumlah 20 haplotip dicerap, di mana haplotip AAAA dan BAAA menunjukkan frekuensi yang paling tinggi. Purata frekuensi haplotip AAAA adalah 29.93% manakala purata frekuensi haplotip BAAA adalah 27.55%. Haplotip BDAB adalah unik bagi populasi Sungai Sefid Rud, haplotip ADAA dan ABAC adalah unik bagi populasi Sungai Lamir, haplotip BAAB adalah unik bagi populasi Sungai Shir Rud manakala haplotip BAAC adalah unik bagi populasi Tajan. Berdasarkan kepada kaedah penunjuk RFLP, empat populasi Mahisefid dibahagikan kepada 2 kluster utama, iaitu Populasi Sungai Shir Rudd an Lamir dalam satu kumpulan, manakala populasi Sungai Tajan dan Sungai Sefid Rud dalam kumpulan lain. Pengkelompokan ini tidak mengikut jarak geografi bagi keempat-empat sungai tersebut.

Dalam kajian mikrosatelit, 120 spesimen (30 sampel dari setiap sungai) *R. frissi Kutum* digunakan. Daripada 30 primer mikrosatelit yang digunakan, 8 primer adalah

polimorfik. Keheterozigotan di antara lokus bagi semua sampel berbeza dari 0.13 hingga 0.91. Untuk lokus tertentu, heterozigot cerapan sangat berbeza antara semua sampel yang dikaji. He tertinggi ialah 0.92 pada lokus SYP4 bagi populasi Sungai Shir Rudd dan He terendah ialah 0.24 pada lokus SYP6 bagi populasi Sungai Tajan. Nilai F_{st} antara semua populasi menunjukkan perbezaan bererti ($P < 0.05$) yang menunjukkan keempat-empat populasi adalah berbeza yang mana selari dengan jarak geografinya. Terdapat perbezaan ketara ($P < 0.05$) daripada Keseimbangan Hardy Weinberg pada semua lokus dan sungai kecuali pada lokus SPY6. Dendrogram menunjukkan populasi Mahisefid terbahagi kepada dua kluster utama, iaitu populasi Sungai Shir Rud dalam satu kluster manakala 3 populasi lagi dalam kluster yang lain.

Keputusan daripada kaedah morfometrik, penanda RFLP dan mikrosatelit dalam kajian ini perlu diambil perhatian oleh penggubal polisi dan pengurus perikanan untuk program peningkatan stok Mahisefid di Iran. Kajian ini mencadangkan populasi Mahisefid dari sistem sungai yang berlainan sepatutnya diurus secara berasingan kerana ia adalah stok berlainan dan tidak sepatutnya dicampurkan terutamanya semasa menjalankan aktiviti pembiakan aruhan.

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DEDICATION

To my loving wife Masoumeh Esmaeely Moghadam and Mr Mohamad Reza Esmaeely Moghadam whose never-ending support and encouragement helped me to believe in myself and discover that I can complete my degree.



APPROVAL

I certify that an Examination Committee met on Monday 18 October 2010 to conduct the final examination of **Hossein Ali Abdolhay** on his Doctor of Philosophy thesis entitled "Morphometric Analysis and Population Genetics of Mahisefid (*Rutilus frisii kutum* kamensky,1901) in south of Caspian Sea" in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded Doctor of Philosophy.

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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 University Putra Malaysia or other institutions.

HOSSEIN ALI ABDOLHAY

Date: 18 October 2010

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