



UNIVERSITI PUTRA MALAYSIA

***ISOLATION, CHARACTERIZATION AND APPLICATION OF
MICRO-SATELLITE MARKERS IN PERSIAN STURGEON,
ACIPENSER PERSICUS BORODINE***

MEHDI MOGHIM

FBSB 2013 9



**ISOLATION, CHARACTERIZATION AND APPLICATION OF
MICRO-SATELLITE MARKERS IN PERSIAN STURGEON,
ACIPENSER PERSICUS BORODINE**

By

MEHDI MOGHIM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

February 2013

To those that devoted their lives to science, nothing can give more happiness than making discoveries, but these cups of joy are full only when the results of their studies find practical application.

Louis Paster

DEDICATIONS

I would like to dedicate it to my decendent father and mother and my beloved family: brother, sisters and step mother, Thank you for the never ending loves and support. My gratitude goes to my father and mother in-law for their sincere support. Finally, I dedicate this dissertation to my loving wife, Ladan Farazi, and my children Amirhossein and Shahrzad for their never-ending support and encouragement, helps to believe and discovery of myself to finish long journey of PhD degree.

Thank you,

MEHDI MOGHIM

UNIVERSITI PUTRA MALAYSIA

February, 2013

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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February 2013

Chair: Professor Tan Soon Guan, PhD

Faculty: Biotechnology and Biomolecular Sciences

The Persian sturgeon (*Acipenser persicus*) is one of the most valuable genetic resources, which is an endangered species. The development of a conservation program for this species requires knowledge of its genetic diversity obtained by using a reliable molecular marker system to ascertain its population genetic structure. Developing such markers in sturgeons that have polyploid ancestry is particularly challenging because many markers exhibit polysomic inheritance.

For this purpose, several experiments were conducted to develop single locus deoxyribonucleic acid (DNA) microsatellite markers for the Persian sturgeon. An enriched library was prepared based on a modified biotin-capture method. Approximately, 1800 positive clones were screened. Out of these, 68 microsatellites

primer pairs were designed and tested for use in the Persian sturgeon. While none of the loci amplified showed disomic inheritance in the Persian sturgeon, several of them appeared useful for studies of stellate sturgeon (*Acipenser stellatus*), ship sturgeon (*Acipenser nudiventris*), beluga (*Huso huso*) and Russian sturgeon (*Acipenser gueldenstaedtii*).

Another approach namely cross species amplification was then used in the quest for disomically inherited microsatellite loci in the Persian sturgeon. For this purpose, 56 and 38 sets of microsatellite primer pairs developed in shovelnose (*Scaphirhynchus platorynchus*) and lake (*Acipenser fulvescens*) sturgeons respectively were used in this study. Ninety four loci were successfully amplified and thirteen microsatellite loci that were polymorphic disomic loci were identified. This is the first report of disomic loci in the Persian sturgeon.

For inheritance studies of these disomic loci, two F₁ families of 23 and 28 larvae were produced in 1x1 crosses of the Persian sturgeons. Eleven microsatellite loci were used for genotyping parents and their offspring. This study verified the Mendelian inheritance and disomic segregation of all the investigated loci. The microsatellite markers developed and characterized here open a new perspective for generating fundamental data to devise sound conservation strategies for the polyploid Persian sturgeon and to provide assistance to wild stock enhancement programs for the species in the Caspian Sea. The findings obtained in the present study attest to the usefulness of the investigated microsatellites for parentage control in the Persian sturgeon.

To evaluate the genetic diversity of the Persian sturgeons using 11 single locus DNA microsatellite markers, 167 sturgeon fish were randomly collected from Turkmenistan, Russia, Iran and the Sefid-Rud River region. The size number of the population of specific alleles ranged from 3 to 21 alleles. The mean values of observed heterozygosity were 0.56 ± 0.20 , 0.64 ± 0.14 , 0.67 ± 0.16 , and 0.64 ± 0.11 in Turkmenistan, Russia, Sefid-Rud and Iran populations, respectively. It was also found that the observed heterozygosity is lower than the expected heterozygosity. This means that there is still inbreeding in the Persian sturgeon and a proper breeding program is essential to avoid increasing inbreeding in natural stocks. The Analysis of Molecular Variance (AMOVA) of microsatellites revealed that there are variations among populations and among individuals within populations. Cluster analysis using admixture model showed evidence of similarity among all the investigated populations. The present analysis of investigated loci showed low or no genetic differentiation or low structuring across populations at all loci between populations.

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

**PEMISAHAN, PENYIFATAN DAN PENGGUNAAN PENANDA
SATELIT-MIKRO DALAM STURGEON PARSII,
ACIPENSER PERSICUS BORODINE**

Oleh

MEHDI MOGHIM

Februari 2013

Pengerusi: Profesor Tan Soon Guan, PhD

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Sturgeon Parsi (*Acipenser persicus*) adalah antara spesies yang terancam serta mempunyai sumber genetik yang berguna. Pembangunan program pemuliharaan bagi spesies ini memerlukan pengetahuan berkaitan kepelbagaian genetik yang boleh diperolehi melalui penggunaan sistem penanda molekul bagi menentukan struktur populasi genetik. Membangunkan penanda tersebut bagi sturgeon terutamanya, yang mempunyai keturunan poliploid, adalah mencabar kerana banyak penanda yang menunjukkan perwarisan polisomik.

Untuk tujuan ini, beberapa eksperimen telah dijalankan untuk membangunkan penanda mikrosatelit lokus tunggal bagi sturgeon Parsi. 'Perpustakaan-diperkaya' mikrosatelit telah dibina berdasarkan kaedah *Biotin-capture* yang diubahsuai. Kira-kira 1800 klon positif telah diskriminasi. Daripada jumlah tersebut, 68 mikrosatelit

pasangan primer telah dibina dan diuji untuk digunakan dalam populasi sturgeon Parsi. Walaupun tiada lokus teramplifikasi menunjukkan perwarisan secara disomik bagi sturgeon Parsi, beberapa lokus ternyata berguna untuk kajian sturgeon lain seperti 'Stellate sturgeon' (*Acipenser stellatus*), 'Ship sturgeon' (*Acipenser nudiiventris*), 'Beluga' (*Huso huso*) dan 'Russian sturgeon' (*Acipenser gueldenstaedtii*).

Pendekatan lain iaitu amplifikasi antara spesies kemudian dilakukan dalam usaha untuk mendapatkan lokus mikrosatelit yang diwarisi secara disomik dalam sturgeon Parsi. Bagi tujuan ini, 56 dan 38 set pasangan primer mikrosatelit yang dibangunkan bagi 'Shovelnose' (*Scaphirhynchus platorynchus*) dan 'Lake' (*Acipenser fulvescens*) sturgeon masing-masing telah digunakan. Sembilan puluh empat lokus telah berjaya diamplifikasi dan 13 lokus mikrosatelit yang polimorfik serta disomik telah dikenal pasti. Ini merupakan penemuan pertama lokus disomik dalam sturgeon Parsi.

Untuk kajian perwarisan lokus disomik tersebut, dua generasi F₁ dengan jumlah larva sebanyak 23 dan 28 dihasilkan melalui persilangan 1×1 sturgeons Parsi. Sebelas lokus mikrosatelit telah digunakan bagi pencirian genotip induk dan keturunan mereka. Kajian ini telah mengesahkan pewarisan Mendel dan segregasi disomik semua lokus yang dikaji. Penanda mikrosatelit yang telah dibina dan dicirikan ini membuka perspektif baru bagi menjana data asas bagi merangka strategi pemuliharaan untuk sturgeon Parsi yang poliploid serta membantu dalam program-program peningkatan stok spesies liar di Laut Kaspia. Penemuan ini membuktikan kegunaan mikrosatelit yang dikaji bagi pengawalan bakasturgeon Parsi.

Untuk menilai kepelbagaian genetik sturgeons Parsi menggunakan 11 penanda mikrosatelitlokus tunggal, 167 ikan sturgeon dikutip secara rawak dari Turkmenistan, Rusia dan Iran serta rantau-Sungai Sefid-Rud. Bilangan alel spesifik populasi adalah di antara 3-21 alel. Min heterozigositi diperoleh ialah 0.56 ± 0.20 , 0.64 ± 0.14 , 0.67 ± 0.16 dan 0.64 ± 0.11 masing-masing bagi populasi Turkmenistan, Rusia, Sefid-Rud dan Iran. Heterozigositi diperoleh adalah lebih rendah daripada heterozigositi dijangka menunjukkan masih terdapat pembiakbakaan dalam sturgeon Parsi dan program pembiakan yang betul adalah penting untuk mengelakkan pembiakbakaan dalam yang semakin meningkat dalam stok semulajadi spesies ini. Hasil analisis AMOVA menunjukkan wujudnya variasi di antara populasi dan individu dalam populasi manakala analisis kluster menggunakan 'admixture model' pula menunjukkan bukti kesamaan di kalangan semua populasi yang dikaji. Kajian semasa menunjukkan perbezaan genetik serta penstrukturan antara populasi yang rendah pada semua lokus.

Hasil kajian ini adalah penting untuk diambil kira oleh pembuat dasar dan pengurus perikanan untuk program peningkatan stok sturgeon Parsi di Iran.

ACKNOWLEDGEMENTS

First, I would like to thank the Almighty God for his perpetual divine assistance without which nothing could have been achieved. Regardless of any achievement in this scope, there are always many people to thank for their assistance and advice.

I would like to express my gratitude to Professor Dr. Tan Soon Guan who accepted the challenge of supervising a student who had spent most of the time away from UPM. My sincere appreciation goes to Professor Dr. Mohammed Pourkazimi, Professor Dr. Siti Shapor Siraj, and Professor Dr. Jothi M. Panadam for their comments and suggestions.

Sincere thankfulness should be dedicated to Dr. Edward J Heist for his assistance in all aspects of the project. Actually, Dr. Edward Heist was not officially my supervisor, but he kindly supported me with the admission to access his laboratory for almost nine months and generously provided me all facilities and laboratory instrument during my study and therefore I could be able to conduct most of Laboratory work of two main studies of my PhD research in his laboratory at Southern Illinois University of USA.

I would like also to thank Assoc. Professor Reza Pourgholam who provided most of my requested laboratory facilities in Caspian Sea Ecology research center and supported me to do the project and I deeply thank Mohamad Reza Hosseini head of the Iranian fishery trade company due to issuing CITES license for transporting fin

clips samples of Persian sturgeon to USA and also Dr. Toraj Valinasab, Davod Kor, Mohamad Reza Behroze Khoshghalb, Faramarez Bagherzadeh , for assistant to deliver samples to the USA.

During my memorable trip to USA, Aaron Schrey sharing his lab experience and time for helping me in my projects. I will never forget the assistance provided to me by captains and crew of Gilan, Kaspia and Seasara research vessels for data collection and samples from the Caspian Sea during all Caspian Marin Expeditions. I also want to use the opportunity to thank my colleagues and head of SHAHID MARJANI sturgeon fish hatchery due to their assistant in collecting samples of parents and offspring's for my genetic inheritance study.

My sincere appreciation also goes to Associate Professor Dr. Motalebi, director of Iranian Fisheries Research Organization, and his deputies for their financial support to carry out this research project. A special thank goes to my dear friends Davod Kor, Hossein ali Abdolhay, Arash Javanmard and Koroush Khaledi who never leaves me alone and shares their valuable knowledge and experience about analysis of data.

Finally, I would like to acknowledge the moral support of others, and particularly, my wife who has sacrificed her time and treated my family in the best way when I was away from my country. In addition, thanks for all those who have contributed in one way or another towards the success of this research project.

I certify that an Examination Committee met on 20 February 2013 to conduct the final examination of **Mehdi Moghim** on his Doctor of Philosophy thesis entitled " ISOLATION, CHARACTERIZATION AND APPLICATION OF MICRO-SATELLITE MARKERS IN PERSIAN STURGEON, *Acipenser persicus* Borodine" 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 Doctor of Philosophy.

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DECLARATION

I 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, and is not currently, submitted for any other degree at University Putra Malaysia or other institutions.



MEHDI MOGHIM

Date: 20 February 2013

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