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POPULATION AND PHYLOGENETIC STUDIES ON ASIAN
AROWANA (*SCLEROPAGES FORMOSUS*)

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Table of Contents

	Page
Acknowledgements	ii
Table of Contents	iii
List of Tables	ix
List of Figures	x
List of Abbreviations and Symbols	xii
Abstract	xiv
Abstrak	xv
Chapter I	
Introduction	1
1.1 The Ornamental Fish Industry in Malaysia	1
1.2 Arowana Trade	3
1.2.1 Myth of Arowana	3
1.2.2 Background of CITES	4
1.2.3 Arowana and CITES	4
1.3 Application of Genetics in Arowana Stock Management	5
1.4 Goals of This Study	6
Chapter II	
Literature Review	7
2.1 Arowana	7
2.1.1 Classification of Arowana	7
2.1.2 Distribution of Asian Arowana	8
2.1.3 Habitat of Arowana	8
2.1.4 Morphology	9

2.1.5	Diet	9
2.1.6	Breeding	10
2.1.7	Raising The Larvae	11
2.1.8	Strains of Arowana	11
2.1.8.1	Malaysian Gold Arowana	11
2.1.8.2	Indonesian Red Arowana	12
2.1.8.3	Green Arowana	12
2.1.8.4	Indonesian Red-tail Gold Arowana	13
2.2	Application of Molecular Genetic Markers	13
2.3	Molecular Genetic Markers	14
2.3.1	Isozymes	14
2.3.2	Mitochondrial DNA (mtDNA)	15
2.3.2.1	Background of MtDNA	15
2.3.2.2	Application of MtDNA Analysis	15
2.3.2.3	The Drawback of MtDNA	17
2.3.3	Nuclear DNA (nDNA)	18
2.4	DNA Markers Systems	18
2.4.1	Restriction Fragment Length Polymorphism (RFLP)	18
2.4.2	Random Amplified Polymorphic DNA (RAPD)	19
2.4.3	Microsatellites	20
2.4.3.1	Background of Microsatellites	20
2.4.3.2	Application of Microsatellites	21
2.4.3.3	Microsatellite Evolution	23

	2.4.3.4 Controversy of Microsatellite	25
	2.4.3.4.1 Null allele	25
	2.4.3.4.2 Size homoplasy	25
	2.4.3.4.3 Shadow bands	26
Chapter III	Materials and Methods	28
	3.1 Sample Collection	28
	3.2 Genomic DNA Extraction	29
	3.3 Microsatellite Loci Analysis	30
	3.4 Mitochondrial DNA Analysis	32
	3.5 Data Analysis	34
	3.5.1 Microsatellite Variation	34
	3.5.2 Bottleneck	36
	3.5.3 Mitochondrial Analysis	37
Chapter IV	Results	38
	4.1 DNA Yield	38
	4.2 Micorsatelite Variation	39
	4.2.1 Locus <i>D01</i>	39
	4.2.2 Locus <i>D04</i>	39
	4.2.3 Locus <i>D11</i>	43
	4.2.4 Locus <i>D13</i>	43
	4.2.5 Locus <i>D14</i>	45
	4.2.6 Locus <i>D15</i>	46
	4.2.7 Locus <i>D16</i>	48
	4.2.8 Locus <i>D27</i>	49

4.2.9	Locus <i>D31</i>	49
4.2.10	Locus <i>D32</i>	51
4.2.11	Locus <i>D33</i>	53
4.2.12	Locus <i>D35</i>	53
4.2.13	Locus <i>D37</i>	54
4.2.14	Locus <i>D38</i>	56
4.2.15	Locus <i>D42</i>	58
4.2.16	Locus <i>D72</i>	58
4.2.17	Locus <i>D85</i>	58
4.2.18	Locus <i>D88</i>	62
4.2.19	Locus <i>D92</i>	62
4.2.20	Locus <i>D94</i>	64
4.2.21	Locus <i>D95</i>	66
4.3	Comparison between Different Laboratory Procedures	67
4.4	Genetic Variability Among Loci	69
4.5	Genetic Diversity Among Strains	69
4.6	Hardy-Weinberg Equilibrium	79
4.7	Linkage Disequilibrium	86
4.8	Bottleneck Analysis	86
4.9	Genetic Differentiation Among Strains	91
4.10	MtDNA Sequence Variability	94
4.11	Strains Differentiation	95
Chapter V	Discussion	101

5.1.1	Conservation of Microsatellites	101
5.1.2	Variability of Microsatellite Loci	102
5.1.2.1	Mutation Rate in Different Types of Microsatellite	102
5.1.2.2	Correlation between Mutation Rate of A Microsatellite and Its Length	102
5.1.2.3	Effect of Different Procedures	103
5.1.3	Loss of Genetic Variability	103
5.1.4	Hardy-Weinberg Equilibrium	104
5.1.5.1	Population Bottleneck	106
5.1.5.2	Importance of Genetic Management in Arowana Conservation	109
5.2	MtDNA Variation	110
5.2.1	Transitional and Transversional Bias	110
5.2.2	Conservation of Amino Acid Sequence	111
5.3	Genetic Differentiation	112
5.3.1	Correlation between Molecular Markers and Geographic Regions	112
5.3.2	Correlation between Molecular Markers and Quantitative Trait Variation	113
5.3.3	Genetic Differentiation between Strains	114
5.4	Comparison of Microsatellite and MtDNA]	115
5.5	Biogeography of Arowana	116
5.5.1	Origin of Asian Arowana	116
5.5.2	Dispersal and Divergence of Arowana in South-east Asia	117
5.5.3	Evolution of Arowana	121

	5.6 Speciation of Arowana	122
Chapter VI	Conclusion	124
References		126
Appendix	1 Breeding Arowana in Captive Environment	142
	2 Arowana Varieties	146
	3 Methodological Outlines	148
	4 Phenotypes of Seven Strains of Arowana in Twenty-one Loci	150
	5 Sequences of <i>ATPase8</i> and <i>ATPase6</i>	165
	6 GenBank Accession Number of Each Haplotype	170

List of Tables

Table	Page
3.1 Varieties, sampling sites and sample size of arowana	28
3.2 Primer sequences of 21 microsatellite loci	30
3.3 Annealing temperature of each locus	32
4.1 Different fragment sizes on MetaPhor agarose and polyacrylamide gels	68
4.2 Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana	71
4.3 P-value of the arowana strains to the Hardy-Weinberg expectation	81
4.4 Observed gene diversity, equilibrium gene diversity and excesses or deficiencies in heterozygosity under the two-phased model of mutation and the assumption of equilibrium effective population size	87
4.5 Pairwise comparisons of shared microsatellite alleles and F_{ST} values between 7 strains of arowana	92
4.6 Variable sites for <i>ATPase8</i> and <i>ATPase6</i>	97
4.7 <i>ATPase</i> base variability by codon position	98
4.8 Results of analysis of molecular variance (AMOVA)	98
4.9 Pairwise mtDNA sequence divergence	99

List of Figures

Figures	Page
4.1 Genomic DNA of Asian Arowana obtained from scales using TNES-Urea buffer	38
4.2a Microsatellite polymorphisms detected in locus <i>D01</i>	40
4.2b Microsatellite polymorphisms detected in locus <i>D01</i>	41
4.3a Microsatellite polymorphisms detected in locus <i>D04</i>	42
4.3b Microsatellite polymorphisms detected in locus <i>D04</i>	42
4.4 Microsatellite polymorphisms detected in locus <i>D11</i>	44
4.5 Microsatellite polymorphisms detected in locus <i>D13</i>	45
4.6 Microsatellite polymorphisms detected in locus <i>D14</i>	46
4.7 Microsatellite polymorphisms detected in locus <i>D15</i>	47
4.8 Microsatellite polymorphisms detected in locus <i>D16</i>	48
4.9 Microsatellite polymorphisms detected in locus <i>D27</i>	50
4.10 Microsatellite polymorphisms detected in locus <i>D31</i>	51
4.11 Microsatellite polymorphisms detected in locus <i>D32</i>	52
4.12 Microsatellite polymorphisms detected in locus <i>D33</i>	53
4.13 Microsatellite polymorphisms detected in locus <i>D35</i>	54
4.14a Microsatellite polymorphisms detected in locus <i>D37</i>	55
4.14b Microsatellite polymorphisms detected in locus <i>D37</i>	56
4.15 Microsatellite polymorphisms detected in locus <i>D38</i>	57
4.16 Microsatellite polymorphisms detected in locus <i>D42</i>	59
4.17 Microsatellite polymorphisms detected in locus <i>D72</i>	60
4.18 Microsatellite polymorphisms detected in locus <i>D85</i>	61

4.19	Microsatellite polymorphisms detected in locus <i>D88</i>	63
4.20	Microsatellite polymorphisms detected in locus <i>D92</i>	64
4.21	Microsatellite polymorphisms detected in locus <i>D94</i>	65
4.22	Microsatellite polymorphisms detected in locus <i>D95</i>	66
4.23	Microsatellite polymorphisms detected in locus <i>D27</i> on polyacrylamide gel	6
4.24	Neighbor-joining (NJ) tree of arowana based on microsatellite	9
4.25	MtDNA fragment of <i>ATPase6</i> and <i>ATPase8</i>	94
4.26	Neighbor-joining (NJ) tree of mtDNA <i>ATPase</i> haplotypes of the arowana	100
5.1	Location map of the contemporary river systems adapted from Verstappen (1975)	120

List of Abbreviations and Symbols

Chemicals

EDTA	Ethylenediamine tetraacetate
MgCl ₂	Magnesium chloride
SDS	Sodium dodecyl sulphate
TBE	Tris-borate-EDTA buffer

Symbols

bp	Base pair
°C	Celsius
F_{IS}	Inbreeding coefficient
F_{ST}	Genetic distance (number of different alleles)
ϕ_{ST}	Genetic distance (number of different nucleotides)
H_e	Expected heterozygosity
H_o	Observed heterozygosity
H_{eq}	Equilibrium gene diversity
kb	Kilo base pair
min	Minute
mM	Mili molar
ng	Nano gram
OD	Optical density
MYA	Million years ago
s	Second

μg	Microgram
μl	Micro litre
μM	Micro molar
V	Volt
w/v	Weight/volume

ABSTRACT

The Asian arowana (*Scleropages formosus*) is an extremely popular and expensive aquarium fish. This species is endangered in the wild and was listed in CITES Appendix I. Genetic variation within and between various two wild and five hatchery strains of arowana were assessed by means of microsatellite analysis. MtDNA sequences were used to infer the phylogenetic relationships of arowana. The twenty one microsatellite loci screened showed different degrees of polymorphisms. Null alleles were detected frequently in the Malaysian red-tail gold arowana. A reduction of microsatellite heterozygosity was observed. Every strain screened in this study faced loss of microsatellite alleles and a different degree of inbreeding. A recent bottleneck was detected in the wild and hatchery strains of green arowana. The microsatellite data yielded high value of F_{ST} between strains except for between the wild and hatchery strains of the Malaysian yellow-tail gold arowana. There were weak correlations between microsatellite markers with morphological traits and with geographical regions. The green arowana is seen as the outgroup while the Malaysian red-tail gold has a closer relationship with the farmed green arowana than with the farmed Malaysian yellow-tail gold and the Indonesian gold arowana. Sequences of *ATPase* subunit 6 and 8 were obtained for ten specimens from five colour strains and all the haplotypes had unique sequences. The sequences of the *ATPase* gene of the arowana were not as variable when compared with microsatellites. The mtDNA data yielded a gene tree with a topology different from that based on microsatellites. The arowana is made up of a monophyletic group of mtDNA with three different lineages which represent the three different colours red, green and gold. The red arowana is the outgroup but the phylogeny is not fully resolved for the gold strains. The phylogenetic structure derived from mtDNA was correlated with colour trait but was not associated with geographical regions. Using the molecular clock for fish *ATPase* genes, the divergence time of the different colour strains of arowana was dated back to from the late Pliocene to the late Pleistocene eras. Based on the molecular and paleogeographical evidences, it is believed that the arowana dispersed in South East Asia when Sundaland was formed even though their migrational pathway is still yet to be elucidated.

ABSTRAK

Arowana Asia (*Scleropages formosus*) merupakan ikan akuarium yang sangat mahal. Spesies ini terancam di persekitaran semula jadi dan disenaraikan dalam Apendiks I CITES. Analisis mikrosatelit digunakan untuk menilaikan variasi genetic daalm dan antara dua strain liar dan lima strain ternakan arowana. Urutan nukleotid mtDNA digunakan untuk menentukan hubungan filogenetik arowana. Alel null kerap dikesan dalam arowana emas Malaysia yang berekor merah. Pengurangan keheterozigotan dikesan. Semua strain arowana menghadapi pengurangan alel mikrosatelit dan tahap pembiakbakaan dalam yang berbeza. "Bottleneck" yang terjadi baru-baru ini dikesan dalam strain arowana hijau dari kawasan semula jadi dan lading. Data mikrosatelit menghasilkan nilai F_{ST} yang tinggi di antara setiap strain kecuali antara strain emas Malaysia yang berekor kuning. Korelasi antara penanda mikrosatelit dengan ciri-ciri morfologi dan kawasan geografi adalah lemah. Arowana hijau merupakan kumpulan terasing. Araoana emas Malaysia yang berekor merah pula berhubung rapat dengan arowana emas Malaysia yang berekor kuning dan arowana emas Indonesia. Sepuluh spesimen yang terdiri daripada lima strain dipilih untuk memperoleh urutan nukleotid subunit 6 dan 8 *ATPase*. Semua haplotip mempunyai urutan yang unik. Variasi gen *ATPase* adalah lebih rendah berbanding dengan mikrosatelit. Data mtDNA menghasilkan dendrogram yang berbeza dengan mikrosatelit. Spesies ini merupakan kumpulan monofiletik dengan tiga asal usul yang mewakili tiga warna, iaitu merah, hijau dan emas. Arowana merah merupakan kumpulan terasing tetapi hubungan antara strain emas tidak dapat ditentukan. Struktur filogenetik yang diperolehi daripada mtDNA berkorelasi dengan ciri-ciri warna tetapi tidak berkaitan dengan kawasan geograafi. Pencapahan antara strain arowan yang berlainan dijangka berlaku dari lewat Pliocene hingga lewat Pleistocene. Berdasarkan bukti molekul dan palcogeografi, arowana dipercayai tersebar di Asia Tenggara apabila Sudaland terbentuk.