

Investigation on Genetic Diversity of Three *Clarias macrocephalus* Populations Using Sequence Of MtDNA D-loop Gene Region

Name : Nazia Bt Abdul Kadar

Supervisor : Dr. Siti Azizah Mohd Nor

School of Biological Sciences, Universiti Sains Malaysia, Penang,
Malaysia.

Co-supervisor : Dr. Thuy Thi Thu Nguyen

Network of Aquaculture Centres in Asia-Pacific (NACA) Thailand.

- Introduction
- Objectives
- Materials & Methods
- Results and Discussion
- Conclusion

Introduction

- *Clariidae* - Airbreathing Catfishes
- *Clarias macrocephalus* - Günther, 1864
- Local name : Keli bunga / keli kampong
- Habitat - wetlands and rivers
- *Clarias* - important cultured fish group
- Exotic *Clarias gariepinus* - high growth rate
- hardiness



Plate 1. *Clarias macrocephalus*

- DNA markers –
 - Species / strain identification
 - Genetic variability
 - Inbreeding
 - Parentage assignment
- Mitochondrial DNA (mtDNA) - population genetics
- D-loop - Control region / displacement loop
- Conserved / highly variable

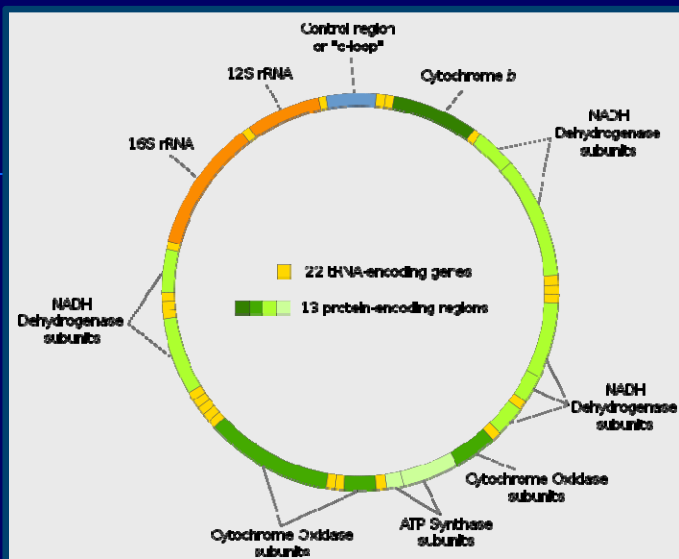


Fig. 1. Mitochondrial DNA.

http://en.wikipedia.org/wiki/Mitochondrial_DNA

Objectives

- To investigate genetic variation of *C. macrocephalus* from Malaysia

Materials & Methods

Sampling sites



Fig. 2. Map of Peninsular Malaysia

DNA extraction



Plate 2. *C. macrocephalus*

PCR amplification

94°C for 2 min
 29 cycles (94°C for 1 min,
 56 for 1 min 10 sec and
 72°C for 2 min)
 final extension
 of 5 min at 72°C.



Plate 3. Peltier Thermal Cycler (PTC-200).

Purification & Sequencing
 (ABI3730XL Genetic Analyzer)



Plate 4. Syngene Bio Imaging

Data analysis

- Alignment: Clustal W Program (MEGA 4.0)
- Haplotype identification: Collapse 1.2 software (Posada, 2006)
- Diversity indices and population differentiation: Arlequin version 3.1 (Excoffier and Schneider, 2005)
- Minimum spanning network: Network ver 4.5

Results & Discussion

- Total of 63 wild individuals of *C. macrocephalus* from Kedah (n=21), Perlis (n=21) and Kelantan (n=21) were sequenced.
- 417 bp partial sequence of mtDNA D-loop gene was successfully amplified.

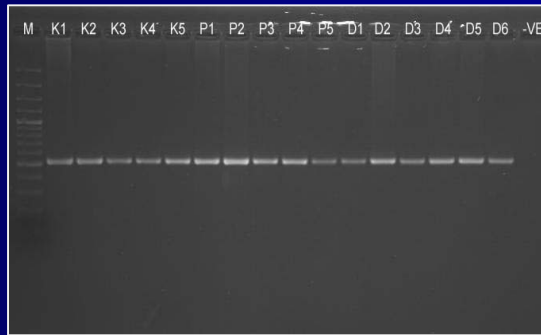


Plate 5. 1.7% agarose gel of PCR products amplified by D-loop mtDNA gene. M: 100 bp DNA Ladder Plus, K1-K5 Kedah, P1-P5 Perlis and D1-D6 Kelantan. -VE: negative control.

Table 1. Alignment of variable sites of 12 haplotypes found among three populations of *C. macrocephalus*.

Haplotype	1	1	2	2	2	2	2	2	3	3	3	4	4	4	4	4	4	4
	6	8	2	4	5	6	6	9	0	4	5	0	0	1	1	1	1	1
	6	5	0	2	8	7	9	6	7	4	7	7	8	0	1	2	4	5
Hap01	C	C	A	T	G	T	G	G	C	A	T	A	G	A	T	C	A	G
Hap02	A
Hap03	T	.	.	C	A	C	.	A	T
Hap04	T	.	.	C	A	C	.	A	T	G
Hap05	T
Hap06	T	A
Hap07	G
Hap08	T	T	C	C	A	C	.	A	T	G
Hap10	T	C	.	.	G	.	.
Hap11	C	T	A	T	G	A
Hap12	T	.	.	.	G	A

Table 2. Number of haplotypes found in all three populations of *C. macrocephalus*.

Haplotype	Population			Total
	K	P	D	
Hap01	13	14	14	41
Hap02	4	3	1	8
Hap03	1	1		2
Hap04	1		3	4
Hap05	1	1		2
Hap06	1			1
Hap07		1		1
Hap08		1		1
Hap09			1	1
Hap10			1	1
Hap11			1	1
Hap12		1		1

Table 3. Haplotype frequencies, number of samples, nucleotide and haplotype diversities, number of haplotypes and polymorphic sites amongst populations of *C. macrocephalus*.

	K	P	D
Hap01	0.619	0.667	0.667
Hap02	0.19	0.143	0.048
Hap03	0.048	0.048	
Hap04	0.048		0.143
Hap05	0.048	0.048	
Hap06	0.048		
Hap07		0.048	
Hap08		0.048	
Hap09			0.048
Hap10			0.048
Hap11			0.048
Hap12		0.048	
Number of samples	21	22	21
Nucleotide diversity (Π)	0.004	0.004	0.017
Number of haplotypes	6	6	6
Haplotype diversity (h)	0.600	0.552	0.552
Number of polymorphic sites	8	11	62

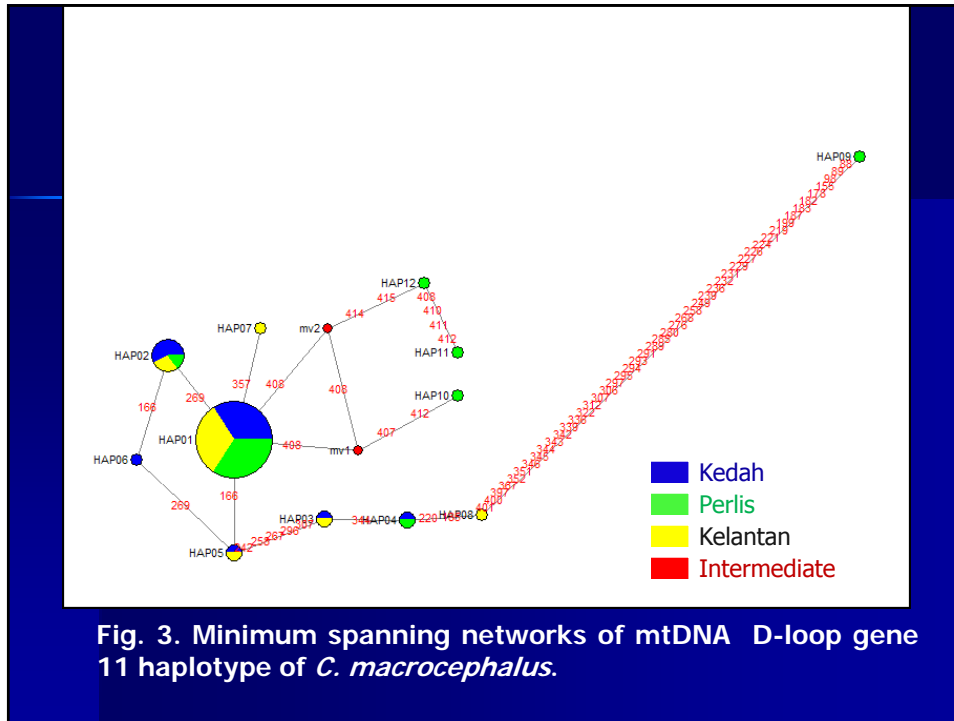


Table 4. Pairwise F_{ST} value (above diagonal) and population differentiation p-value (below diagonal) based on 417 bp sequence data for *C. macrocephalus*.

	Kedah	Perlis	Kelantan
Kedah		-0.037	-0.01
Perlis	0.999		-0.01
Kelantan	0.5361	0.5098	

Conclusion

- All three populations studied were homogenous
- Several population-specific haplotypes suggest that the populations are undergoing independent evolution
- Genetic data obtained from this study serve as an important tool for the conservation and management of this species

THANK YOU

- D-loop Primer : H355 5'-CCT-GAA-ATG-AGG-AAC-CAG-ATG-3'
L16473 5'-CTA-AAA-GCA-TCG-GTC-TTG-TAA-TCC-3'.