

MITOCHONDRIAL DIVERSITY OF THE KELI BUNGA, *Clarias macrocephalus* (GÜNTHER, 1864), INVESTIGATED USING D-LOOP AND ND5 GENES.

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ABSTRACT

Genetic variation of six *Clarias macrocephalus* (Günther, 1864) populations were assessed using mitochondrial genes, D-loop (417 bp) and dehydrogenase subunit five (ND5) (1004 bp). 152 individuals were sequenced for D-loop and 80 individuals for ND5. The analyses revealed 32 and 18 haplotypes for D-loop and ND5, respectively. Based on F_{ST} values, all the populations were significantly different from each other except for Kedah, Perlis and Kelantan.

KEYWORDS: *Clarias macrocephalus*, D-loop, ND5, mtDNA genes

INTRODUCTION

The genus *Clarias* is found in tropical climate regions, such as in Southeast Asia and East Asia westwards through India and Africa (www.fishbase.org). According to Mohsin & Ambak (1983) and Lee *et al.*, (1993), *C. macrocephalus* (Günther, 1864) also known as keli bunga or keli kampong locally, can be found in North-West (Kedah, Perak), North-East (Terengganu) and Central (Melaka) Peninsular Malaysia. The genus is an important cultured fish group in Malaysia and their production in 2004 alone was valued at RM 39 million (Department of Fisheries, 2004). However, wild populations are fast depleting because of habitat competition from *C. gariepinus* as well as fishing activities and also genetic introgression from cultured escapees. *Clarias macrocephalus* can easily hybridise with *C. gariepinus* in the wild. In fact in the late 1980s, Thailand successfully produced hybrids of *C.*

macrocephalus and *C. gariepinus* and the technology was implemented by local farmers to produce hybrids which possessed the advantageous qualities of keli bunga. These hybrids soon dominated production. This study reports on the utilisation of mtDNA markers to investigate the genetic variation of *C. macrocephalus* as a strategy for the conservation and breeding programmes of this species.

OBJECTIVES

The objectives of this study were to investigate genetic variation of *C. macrocephalus* populations using mtDNA genes and to develop a genetic baseline data of catfish to be utilized in aquaculture and biodiversity conservation programmes.

MATERIALS AND METHODS

Wild samples (Table 1) were collected from its distributional range in Peninsular Malaysia. A Vietnamese population was also included. Genomic DNA was extracted from the dorsal or caudal fin by using AquaGenomic™ Kit (MultiTarget Pharmaceuticals, Salt Lake City, Utah, USA) for PCR amplification.

Sampling site	Coordinate (North)	Coordinate (East)	Sample codes
Kedah (Kuala Nerang)	6 ⁰ 14'03.84''	100 ⁰ 36'10.45''	K
Perlis (Arau)	6 ⁰ 25'32.95''	100 ⁰ 19'32.93''	P
Kelantan (Tanah Merah)	5 ⁰ 48'39.08''	102 ⁰ 23'47.42''	D
Pulau Pinang (Seberang Prai)	5 ⁰ 23'34.12''	100 ⁰ 23'34.12''	R
Terengganu (Dungun)	4 ⁰ 44'29.07''	103 ⁰ 22'03.35''	TB
Vietnam (Mekong River)	12 ⁰ 13'18.68''	105 ⁰ 32'16.69''	V

* Details of sample numbers as in Tables 2a & 2b

Table 1. Sampling site coordinates and sample codes.

D-loop (417 bp) and ND5 fragments (1004 bp) were successfully amplified for all samples. Primers used for D-loop amplifications were H355 5'-CCT-GAA-ATG-AGG-AAC-CAG-ATG-3' and L16473 5'-CTA-AAA-GCA-TCG-GTC-TTG-TAA-TCC-3' (Imsiridou *et al.*, 1998) and for ND5 amplifications were L12321 5'-GGTCT-TAGGAA-CCCAAAA-CTCTT-GCTGCAA-3' and H13396 5'-CCTA-TTTTK CGGAT-GTCYTG-3' (Miya & Nishida, 2000). Amplification was performed in a 25µl reaction containing 5X PCR Buffer, 25mM MgCl₂, 10mM dNTP, 5µM of each primer, 25ng of template DNA, 5U of *Taq* DNA Polymerase (Promega, Corporation, Madison, USA) and sterile deionized water in a Peltier Thermal Cycler (PTC-200). The PCR program was 94^oC for 2 min, followed by 29 cycles of 94^oC for 1 min, 56^oC for 1 min 10 sec and 72^oC for 2 min for D-loop. ND5 denaturation was conducted at 95^oC for 2 min, followed by 29 cycles of 95^oC for 1 min, 55^oC for 1 min and 72^oC for 1 min. A final extension of 5 min was conducted at 72^oC for both amplifications. PCR products were purified using Wizard ® SV Gel and PCR Clean-Up System (Promega, Corporation, Madison, USA) and sequenced on an ABI3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were aligned using ClustalW and population analyses performed using Collapse 1.2, Arlequin version 3.11, and finally Network 4.516.

RESULTS AND DISCUSSION

The Terengganu population (TB) was the most variable (Table 2) compared to other populations. This is likely due to the presence of hybrids, presumably between *C. gariepinus* and *C. macrocephalus* either from the wild or escapees from nearby culture facilities. The genetic distances ranged from 0.0083 to 0.1304 and from 0.0012 to 0.1033 for D-loop and ND5 genes respectively. The higher distances were contributed by intraspecific comparisons with TB. Based on pairwise comparisons, ($P > 0.05$), all F_{ST} values were significantly different except for Kedah, Perlis and Kelantan. UPGMA and NJ analyses performed based on Kimura 2-parameter showed that haplotypes were intermixed including the Vietnam population with the exception of haplotypes from Terengganu which formed a different clade. Haplotype 1 was found in all the populations from Malaysia. However, 26 and 15 haplotypes (Accession Number FJ495103-FJ495117) occurred as private population-specific haplotypes for D-loop and ND5 respectively. The minimum spanning network analysis shows the evolutionary relationships among haplotypes. Presence of several population-specific haplotypes suggests that the populations had undergone independent evolution and as such should be conservatively managed as separate management units.

Population	K	P	D	R	TB	V
Number of samples	33	32	25	14	26	22
Nucleotide diversity (π)	0.010	0.006	0.016	0.021	0.080	0.013
Number of haplotypes	7	8	8	4	10	8

Haplotype diversity (h)	0.670	0.657	0.637	0.736	0.827	0.865
Number of polymorphic sites	47	11	60	42	107	15

Table 2(a). Number of samples, nucleotide and haplotype diversities, number of haplotypes and polymorphic sites amongst populations of *Clarias macrocephalus* based on D-loop gene

Population	K	P	D	R	TB	V
Number of samples	20	20	20	7	4	9
Nucleotide diversity (π)	0.014	0.002	0.003	0.000	0.092	0.007
Number of haplotypes	6	3	3	1	4	8
Haplotype diversity (h)	0.447	0.278	0.279	0	1	0.972
Number of polymorphic sites	135	12	12	0	170	20

Table 2(b). Number of samples, nucleotide and haplotype diversities, number of haplotypes and polymorphic sites amongst populations of *Clarias macrocephalus* based on ND5 gene

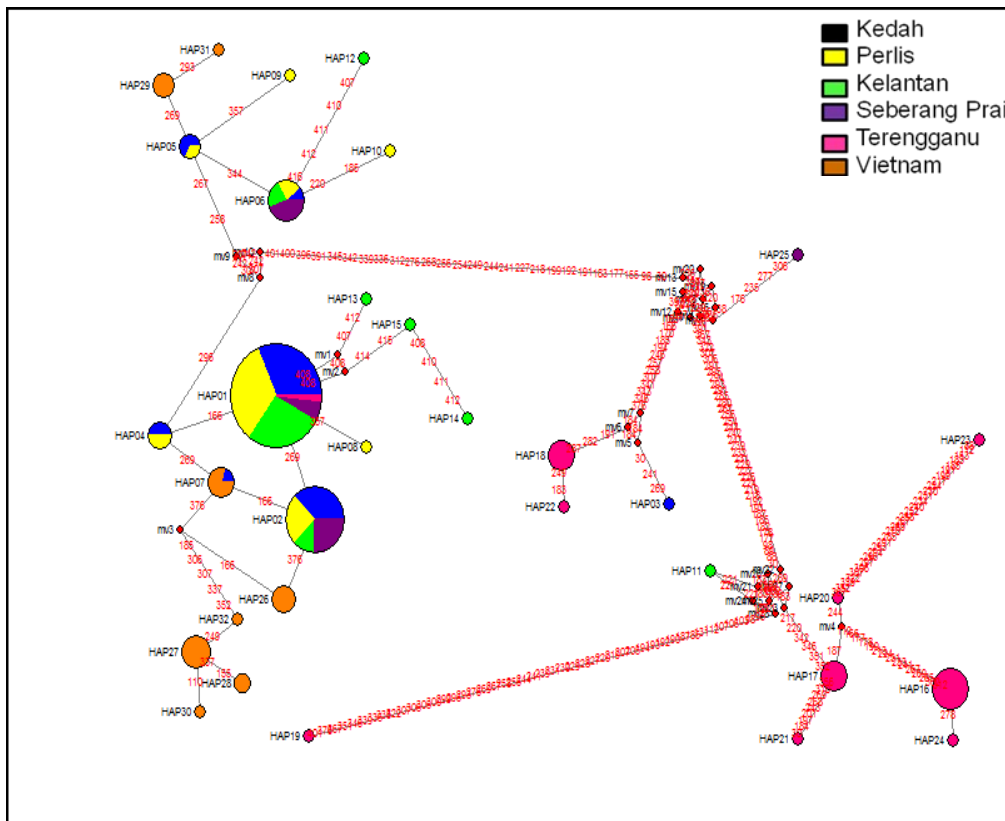


Figure 1. Minimum spanning network of mtDNA D-loop gene.

CONCLUSION

Understanding the levels of genetic diversity would provide management guidelines for commercial use and conservation of this species.

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