POPULATION GENETIC STRUCTURE OF *Clarias teijsmanni* (Family: Claridae)

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Declaration

I hereby declare that this thesis is originally my own effort and work. It is has not been submitted in any institution or other higher learning. Where all the information that have been, they have been acknowledged.

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List of abbreviation

COI	Cytochrome c oxidase 1
˚C	Celcius
µl	Microliter
mL	Milliliter
mM	milliMol
$MgCl_2$	Magnesium Cloride
CTAB	hexadecyltrimethyl ammonium bromide
PCR	Polymerase Chain Reaction
EtBr	Ethidium Bromide
%	Percentage
DNA	Deoxynucleoside-5'-triphosphate
rpm	Rotation Per Minute
mtDNA	mitochondrial DNA
dNTPs	Dioxyribonucleotidetriphosphate
%	Percentage
mm	millimeter

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ABSTRACT

Mitochondrial DNA of cytochrome oxidase I (COI-2) gene was used to study the population genetic *Clarias teijsmanni*. A total 682 bp of COI-2 gene from 12 samples were analyzed using molecular technique. Two locations were selected in this study include Kubah National Park population and Gunung Gading National Park population. The gene flow between populations was high. This study showed that the species shared same genetic. This shows that this species moving actively in the study sites. Mitochondrial DNA is useful as genetic marker for population genetic study. This study should be done in future to study the overall population genetic structure of *C. teijsmanni* in Sarawak.

Keywords: population genetic, mitochondrial DNA, cytochrome oxidase I (COI-2),*C. teijsmanni*, gene flow

ABSTRAK

Gen sitokromosidasi I (CO1-2) yang terdapat dalam mitokondrial DNA telah digunakan untuk mengkaji populasi genetic bagi spesis C. teijsmanni. 682 pasangan gen sitokromosidasi I (COI-2) telah dianalisa daripada 12 jumlah sampel C. teijsmanni dengan mengunakan teknik molekul.Dua lokasi telah dikaji iaitu Kubah National Park (KNP) dan Gunung Gading National Park (GGNP). Hasil dari kajian ini menunjukkan bahawa aliran gen antara populasi adalah tinggi. Kajian ini menunjukkan bahawa spesis ini berkongsi genetik. Ini menunjukkan bahawa spesis ini bergerak aktif di dalam kawasan kajian. Mitokondrial DNA adalah berguna sebagai penanda genetic bagi kajian populasi genetik. Kajian ini perlu dilakukan pada masa akan dating untuk mengkaji keseluruhan populasi C. teijsmanni di Sarawak.

Kata Kunci: populasi genetik, mitokondrial DNA, sitokromosidasi (COI-2), aliran gen, C. teijsmanni

CHAPTER 1 INTRODUCTION

Clarias is a genus of catfishes in order Siluriformes of family Claridae. They are known as air breathing catfishes. They were found in inland water, stream and river. Some of catfishes have become pest species where they have been accidently introduced. All catfishes lack normal scales, although some have armour of bony plates covering the body instead of being naked (Steward and Watkinson, 2004). According to Ferraris and Pinna, (1999), catfishes are diverse group of fish representing more than 3000 species, 478 genera and 36 families. Most of catfishes have barbells or 'whiskers' around their mouth which help them to locate foods (Steward and Watkinson, 2004). This family is naturally distributed all over Africa, South and Southeast Asia with the highest genetic diversity found in Africa (Agbebi *et al.*, 2013).

Clarias teijsmanni is classified under family claridae. It is also a predator feed on fishes, birds and amphibians. *C. teijsmanni* looks like eel with cylindrial body, flattened body head, abroad transverse mouth with four pairs of long barbel around it. It also has single dorsal fin which is long but lacks of spines. They usually "walk" in water using their pectoral and pelvic fins. This species is found in small forest streams in the primary forest in slow-moving or still water and are extremely cryptic (Fishbase). *C. teijsmanni* has spots on their body and their colors are brown. This species is active at night because. The status in International Union for Conservation of Nature (IUCN) Red list of this species is not evaluated.

The application of molecular technique has provided new and insight into the taxonomy, population and conservation management of freshwater fish (Smith and Wayne, 1996, Nguyen *et al.,* 2006). According to Ryan and Esa (2006), the molecular markers can provide reliable and consistent results for rapids identification among species. Molecular markers can also provide the levels of genetic variability, levels of gene flow and population subdivision, and understanding the factors contributing to fitness (Vrijenhoek, 1998). The mtDNA has higher stability and occurs in a much higher numbers of copies than nuclear DNA (Prusak *et al.,* 2005).

In many cases behavioral or oceanographic mechanisms may limit large-scale dispersal, even in species that have relatively long pelagic larval durations (Sponaugle *et al.,* 2002, Palumbi and Warner, 2003). The degree to which such populations are supported by passive larval drift from the center of the range versus self-replenishing is not clear. Palumbi (2003) has suggested that even species with a high potential for dispersal may show genetic structure that conforms to and isolation-by-distance model due to limited larval exchange between distant populations.

This study is important for future study of *C. teijsmanni* because taxonomy of this species is still vague. The objective of this study is to examine the genetic structure of *C. teijsmanni.* Besides, this study is also to determine genetic differentiation between populations. The other objective of this study is examining the suitability of COI-2 to study population genetic of *C.teijsmanni.*

CHAPTER 2 LITERATURE REVIEW

2.1 Distribution, morphology and taxonomy of catfishes

Based on Teugels, (2003), clariidae is one of the 31 families belonging to the teleostean order Siluriformes. Some species are distributed in Syria, southern Turkey and throughout southeast Asia, but their diversity is the greatest in Africa (Teugels and Andriaens, 2003). According to Teugels, (2003), *clarias* is the only genus occurring on both African and Asian continents and can be divided into seven subgenera.

Species belonging to the genus *clarias* are freshwater catfishes characterized by their ability to utilize atmospheric air and walk on land for several hundred meters using their pectoral spines (Teugels, 1996). The catfishes have dorsal fin which technically has two spines where the first being very short and forming a locking mechanism for the second spines.

Almost of claridae exploit a wide range of habitats from the stream, rivers and freshwater lakes (Agnese and Teugel, 2005). Some species of the catfishes are known to be poisonous or venomous (Perriere and Gaudey, 2003). The barbells or “whiskers” around their mouth are used to help them to elongate the food in a long distance (Steward and Watkinson, 2004). Each alfactory pit has two nostrils which are for incoming water and the other one is for outgoing water. According to Sutton, (2000), these pits are lined with sensitive tissue wrinkled into a series of folds to provide the maximum surface area for smelling.

C.teijsmanni is classified under family claridae. *C. teijsmanni* has cylindrial body, flattened body head, abroad transverse mouth with four pairs of long barbel around it. It is also have single dorsal fin which is long but lacks of spines. They are walking in water using their pectoral and pelvic fins. This species found in small forest streams in the primary forest in slow-moving or still water and were extremely cryptic (Fishbase). *C. teijsmanni* has spots on their body and their colors are brown. This species is active at night. *C. teijsmanni* is known as walking forest catfish. This species is very lacking in taxonomy. Their distribution is very low, so with this study , it can help to increasing their taxomic.

Table 1 Taxonomy of *C.teijsmanni*

Kingdom	Animalia
Phylum	Chordata
Class	Neopterygii
Order	Siluriformes
Family	Claridae
Genus	Clarias
Species	*Teijsmanni*

2.2 Mitochondrial DNA and COI gene

Mitochondrial DNA (mtDNA) is one of molecular markers that used to study the population genetic. MtDNA has been widely used molecular marker for phylogenetic studies in animals because of its simple genomic structure (Avise, 2004). The mtDNA has higher stability

and occurs in a much higher number of copies than nuclear DNA (Prusak *et al.*, 2005). Besides that, mtDNA has many advantages. It evolves faster than nuclear DNA (Brown *et al.*, 1982). It is probably due to the inefficient replication repair. According to Saccone *et al.*, (1991), different regions of the mitochondrial genome evolve at different rates.

Mitochondrial cytochrome oxidase (COI) gene has gained more attention in developing DNA barcodes for species identification and biodiversity analysis (Arif and Khan, 2009). The DNA barcoding has highlighted the expanding use of the COI as a genetic marker for species identification (Dawnay *et al.*, 2007).

2.3 Population Genetic Study of Freshwater Fishes

Previous study by Daud *et al.* (1989) and Lawonyawut (1995) revealed on genetic variation in the Malay and Thailand population of *Clarias macrocephalus.* Genetic study of red drum employed starch gel electrophoresis of proteins encoded by nuclear genes. According to Ramsey and Wakeman (1987), they surveyed allelic variation at four polymorphic loci among red drum sampled from 12 locations in the northwestern Gulf and two locations along the Atlantic coast. The studies of variety of organisms, including fishes have shown that restriction fragment or sites analysis of mtDNA is more powerful than protein electrophoresis for differentiating subpopulation within species (Avise and Lansman, 1983). The genetics studies remain as something new and interesting field yet there were still questionable numbers of solving species origin, lineage and identification of the species.

There is a study by Yang *et al.*, (2009) are about population genetic structures and geographical differentiation of the Chinese catfish *H.macropterus* (Siluriformes, Bagridae). In this study, they revealed that *H. macropterus* of the upper Changjiang River population and the Zhujiang River population are closely related to each other, while the genetic divergence among population of the three parts of the Changjiang River is significant.

CHAPTER 3 METHODOLOGY

3.1 Study Sites

This field work took place at Gunung Gading National Park (GGNP), Lundu, southeast of Sarawak and Kubah National Park. Gunung Gading National Park has an area of 4,106 hectares. GGNP is located near Lundu, about 80km from west of Kuching. The coordinates of GGNP is N01 42.00'E109 50.20'.Whereas, KNP about 20 km west of Kuching and dominated by sandstone and covers an area of 2,230 hectares. KNP is located near Matang. The coordinate of KNP is N1 35.76' E110 10.85'. KNP had water sources such as stream and waterfall.

The distance between Gunung Gading National Park and Kubah National Park were 65.6 km. Both Gunung Gading and Kubah were cover with forest and rocky stream. These two population were chosen because their streams were connected to each other.

3.2 Samples Collection and Preservation

The samples of *C. teijsmanni* were collected with eight individuals from GunungGading National Park and eight individuals from Kubah National Park, Kuching. The specimens were preserved in alcohol or stored at -20°C until it is used for the genetic analysis. The specimens were identified through their morphological characteristics measurement by using keys which is introduced by Inger and Chin (1962).

3.3 DNA Extraction

The DNA from the fin tissues of *C.teijsmanni* was extracted by using a CTAB method (hexadecyltrimethyl ammonium bromide) introduced by Grewe *et al.*,(1993). About 1-2 cubic mm of the tissues sample were added into each of 1.5 ml microcentrifuge tube that containing 600 µl CTAB buffer. Then, the Proteinase K (10mg/ml) was added into the tubes. The solutions in the tube were mixed and incubate it at 65°C for 2-3 hours until the tissues completely dissolve.

After that, chloroform: isoamly alcohol (24:1) was added into each tube. Each tube for 2 minutes was mixed before centrifuge at 13000 rpm about 10 minutes. After centrifuge about 10 minutes, the only upper layer of the supernatant was taken and the solution was transfer into the newly labeled tube. An equal volume of cold absolute ethanol was added and mixes well. After the solution mixed up, the tube was placed at the beach for a few minutes then, the sample was centrifuge at 13000 rpm for 15 minutes. The ethanol was discarded.

Approximately 70% of cold absolute ethanol with 25 µl 3M NaCl was added into the tube. Then, the tube was centrifuged at 13000 rpm for 15 minutes. The solution was discarded after centrifugations and dries the tube about 15-20 minutes at room temperature. Finally, the DNA pellet was suspended in 30 µl sterile distilled water after the DNA pellet was dried. Then, the DNA extraction was kept in freezer with -20° C.

3.4 Running the Gel Electrophoresis

After DNA extraction, visualize the sample is important to see whether the purification is successful or not. The agarose gel is used to detect whether the band of DNA is good or not. Firstly, weigh the 1% 0.5g of agarose powder into the conical flask. About 50 ml TAE buffer was added into the conical flask then, the solution was added about 2 minutes until the agarose powder completely dissolve in the oven. The 2.0 µl ethidium bromide (EtBr) was added into the solution. The comb was inserted in the casting tray. Then, the agarose solution was poured into the casting tray and cool down for 30 minutes until the gel stiffer. The comb was taken so that the well will appear in the gel. After that, the gel was inserted into the tank with agarose buffer. Then, 2.0 µl of the sample was pipette and it was added to 2.0 µliter of loading gel by adjusting the micropipette to 4 µliter. The mixture was mixed by using the suitable micropipette and inserts it into the well. 2.0 µl of DNA ladder was put in and run at 90 V about 40 minutes. When the band much the third of the bottom line in 30 minutes, it needs to stop. The DNA band was observed under ultraviolet transllumination

3.5 Amplification of DNA by using Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is known as revolution method developed by Mary Mullis in the 1990. PCR is a powerful tool precisely because it can be done using as little as a single or a few copies of template DNA (Hofreiter *et al.,* 2001).

Table 2 Master mixture of PCR:

Components	1X Reaction (µl)
5X PCR Buffer	5.0
$MgCl_2$	1.5
dNTP mix (10mM)	0.5
Primer F (CO1-2) (10mM)	1.0
Primer R (CO1-2) (10mM)	1.0
ddH_2O	13.8
DNA Template	2
Taq Polymerase	0.2
Total Volume	25.0

Table 3 Sequences for CO1-2 Primer (Ivanova *et al.,* 2006)

Primer	Sequences	Direction
COI-2	VF1_t1 5'-TGTAAAACGACGGCCAGTTCTCAACCAACCACA-3'	Forward
COI-2	VR1_t1 5'-CAGGAAACAGCTATGACTAGACTTCTGGGTGGCC-3'	Reverse

Table 4 PCR cycles for *C. teijsmanni* using COI-2 primer

Steps	Temperature	Time	Cycle
Pre-denaturation	94	5	1
Denaturation	94	1	
Annealing	45.7	1	30
Extension	72	1	
Final Extension	72	5	1
Soaking	4	59	

3.6 Phylogenetic Analysis

Visualize sequence result (chromatogram) using CHROMAS program version 1.45 (MacCarthy, 1998). This program will display the fluorescence-based DNA sequence to analyze the result. It will display a chromatogram and show the nucleotide bases. The visualize DNA sequence analysis result WAS done using CLUSTAL X program version 1.81 (Thomson *et al.,* 1997). This program will align the sequences and produce the output files for phylogenetic analysis. Check the stop codon. The phylogenetic tree among haplotypes was done using Molecular Evolutionary Genetic Analysis (MEGA) program version 4.0 (Kumar *et al.,* 2007) to perform Neighbour-Joining (NJ) tree, Maximum Parsimony (MP),

3.7 Population Genetic Structure Analysis

There are two parameters of population genetic diversity measured was used. DnaSP 4.0 (Rozas *et al.,* 2003) was used to calculate indices for haplotypes diversity and nucleotide diversity and nucleotide divergences among *C. teijsmanni* populations. Arlequin ver 3.0 (Excoffier, 2005) was used to find the genetic distance.

3.8 Inferring Gene Flow and Population Expansion

The population expansion was determined through mismatch distribution analysis by using Arlequin ver 3.0. Tajima test of neutrality was used to determine the demographic history of the species.

CHAPTER 4 RESULT

4.1 Sample collection and preservation

Samples of *C. teijsmanni* were collected at Gunung Gading National Park and Kubah National Park. Sixteen samples were collected. The samples were identified by their morphological characters. Then, the samples were preserved in 99% ethanol and placed at 2^{o}C in freezer.

Figure 1: The samples collected after species identification

Picture by: Rosy, 2012

4.2 DNA Extraction

There were eight samples of *C.teijsmanni* collected from Gunung Gading National Park and about eight samples of *C.teijsmanni* were collected from the Kubah National Park.

Table 5 Extraction result for *C. teijsmanni*

Samples ID	Location	Result
GG1	GunungGading National Park,Kuching	DNA band present
GG2	GunungGading National Park,Kuching	DNA band present
KNP1	Kubah National Park,Kuching	DNA band present
GG3	GunungGading National Park,Kuching	DNA band present
GG4	GunungGading National Park,Kuching	DNA band present
KNP2	Kubah National Park,Kuching	No DNA band present
KNP3	Kubah National Park,Kuching	DNA band present
KNP4	Kubah National Park,Kuching	DNA band present
GG5	GunungGading National Park,Kuching	DNA band present
GG6	GunungGading National Park,Kuching	DNA band present
KNP5	Kubah National Park,Kuching	DNA band present
KNP6	Kubah National Park,Kuching	DNA band present
KNP7	Kubah National Park,Kuching	DNA band present
KNP8	Kubah National Park,Kuching	No DNA band present
GG7	GunungGading National Park,Kuching	DNA band present
GG8	GunungGading National Park,Kuching	No DNA band present