MORPHOLOGICAL AND GENETIC VARIABILITY OF MALAYSIAN *CHANNA* SPP BASED ON MORPHOMETRIC AND RAPD TECHNIQUES

NORAINY BINTI MOHD HUSIN

UNIVERSITI SAINS MALAYSIA

2007

MORPHOLOGICAL AND GENETIC VARIABILITY OF MALAYSIAN *CHANNA* SPP BASED ON MORPHOMETRIC AND RAPD TECHNIQUES

by

NORAINY BINTI MOHD HUSIN

Thesis submitted in fulfillment of the requirements for the degree of Masters of Science

FEBRUARY 2007

ACKNOWLEDGEMENTS

Alhamdulillah, syukur ke hadrat illahi kerana dengan izin Nya saya dapat menyiapkan tesis ini.

My sincere gratitude and appreciation to my supervisor Prof. Madya Dr. Siti Azizah Mohd Nor. She has helped me a lot in my research. Through her guidance, patience and encouragement, I have come this far. Thank you Dr.

I also would like to say thank you to K. Za and Abg. Amir, who has taught and guide me at the beginning of my research. My gratitude also goes to other lab mates and friends Fatimah, Emi, Mila, Zam, Azad, Zahir, Abg Amir and Zarul. Thank you also to lab member of Lab 408 (Dr. Sofiman's lab). To other friends who has enriched my life with their friendship and kindness.

My profound gratitude and love to both of my parents for their unconditional love and support. Thank you also to my auntie and uncles for their encouragement.

Last but not least to my husband and my beautiful children; Damia and Adam, I am blessed having you in my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of Variance
DFA	Discriminant Function Analysis
DNA	Deoxyribonucleic Acid
DOF	Department of Fisheries
mtDNA	Mitochondrial DNA
OD	Optical Density
PCA	Principal Component Analysis
PC1	First Principal Component
PC2	Second Principal Component
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RM	Ringgit Malaysia
RNA	Ribonucleic Acid
SNK	Student Newman Keuls
SPSS	Statistical Package for the Social Sciences
TNES urea	Tris-NaCl-EDTA-SDS
UMT	Universiti Malaysia Terengganu
UPGMA	Unweighted Pair-Group Method of Arithmethic

LIST OF APPENDICES

LIST OF PUBLICATIONS & SEMINARS

1.1 **Norainy, M. H**. and M. N. Siti Azizah, 2002. Genetic relationships and variation of five *Channa* species assessed by RAPD technique for conservation purposes. Proceedings of the 4th Indonesia-Malaysia-Thailand Growth Triangle (IMT-GT) UNINET Conference. 15-17th Oct. 2002, Penang, Malaysia. Pp 64-67.

1.2 **Norainy, M. H**. and M. N. Siti Azizah, RAPD fingerprinting snakehead *Channa* sp. for genetic identification in aquaculture. (Submitted to *World Aquaculture Magazine* as Invited Contributor). In prep

1.3 **Norainy, M. H**. and M. N. Siti Azizah, 2002. RAPD fingerprinting of four snakehead *Channa* sp. for genetic identification and aquaculture purpose. Paper presented at International Aquaculture Conference & Exposition "World Aquaculture 2002" April 23-27, 2002. Beijing, China.

1.4 **Norainy, M. H**. and Siti Azizah, M. N., 2003. Analysis of partial mitochondrial region of ND5 gene in six snakehead species, *Channa* from Malaysia. Poster presented at Symposium of Phylogeography of West Pacific, 22-28th Feb. 2003, Taipei, Taiwan.

Morfologi dan kevariabelan genetik *Channa* spp Malaysia berdasarkan teknik morfometrik dan RAPD

ABSTRAK

Satu kajian morfometrik dan genetik telah dijalankan ke atas enam daripada tujuh spesies yang telah didokumenkan di Semenanjung Malaysia; *Channa striata*, *C. gachua*, *C. marulioides*, *C. micropeltes, C. melasoma* and *C. lucius.* Lapan belas ukuran pandangan sisi and 11 ukuran pandangan dorsal telah diperolehi daripada 81 individu. Data telah dianalisis melalui analisis varians, ANOVA dan multivariat (Analisis Komponen Prinsipal – PCA dan Analisis Fungsi Diskriminan –DFA). Berdasarkan statistik ini, pada keseluruhannya, kesemua enam spesies telah terkumpul kepada kelompok spesies tersendiri dengan pertindihan yang minimum. *Channa gachua* didapati yang paling berbeza bagi kedua-dua analisis, kebanyakannya ditunjukkan pada perbezaan saiz pedunkel kaudal (pandangan sisi) dan lebar kepala (pandangan dorsal). Walaupun begitu hubungan antara spesies yang lain berbeza antara kedua-dua analisis. *Channa marulioides*, *C. striata*, *dan C. melasoma* berkelompok dengan rapat sementara *C. lucius* agak jauh sedikit. *Channa micropeltes* terasing berdasarkan jarak antara sirip dorsal ke sirip pektoral pada pandangan sisi. Pada pandangan dorsal *C. striata*, *C. lucius* dan *C. micropeltes* berkait rapat sementara *C. melasoma* berkait rapat dengan *C. marulioides*.

DNA Polimorfik Teramplifikasi Rawak (RAPD) telah digunakan untuk menganggar variasi genetik ke enam spesies tadi dan hubungan genetik antara mereka. Sepuluh wakil bagi setiap spesies telah dianalisis kecuali *C. melasoma* di mana hanya tiga individu diperolehi. Sembilan 10-mer nukleotida primer RAPD iaitu AB-2, AB-7, AB-14, AB-18, F2, F3, F10, F16 dan F20 telah digunakan dalam kajian ini. Kesemua sembilan primer ini mengamplifikasikan 83 lokus yang boleh diskor. Keputusan menunjukkan

variabiliti genetik yang agak tinggi kecuali pada *C. melasoma.* Polimorfisme adalah paling tinggi bagi *C. lucius* (46.7%) dan terendah bagi *C. melasoma* (8.9%) walaupun kedua-duanya adalah simpatrik. Jarak genetik antara perbandingan berpasangan didapati dalam julat 0.6 ke 0.7 walaupun terdapat banyak pertindihan yang diperhatikan antara perbandingan spesies di kalangan semua spesies. Dendogram yang dijana oleh perisian RAPDistance menunjukkan bahawa *C. gachua* merupakan takson yang paling basal. Sementara *C. striata* dan *C. lucius* membentuk takson terminal. Walaupun begitu kedudukan spesies agak berbeza berdasarkan perisian FreeTree tetapi dendrogram yang dihasilkan mempunyai sokongan ”bootstrap interspesies” yang sangat rendah. Namun begitu sokongan bagi monofili setiap spesies adalah sangat baik pada 100%.

Keputusan menunjukkan kegunaan morfometrik dalam pengkelasan spesies dan bagi mentafsirkan perbezaan bentuk dan saiz di kalangan spesies *Channa* . Sebaliknya RAPD boleh digunakan untuk menganggar polimorfisme DNA dan variabiliti intraspesifik. Namun begitu pengaplikasian analisis RAPD untuk kajian sistematik dan filogenetik seharusnya dibandingkan dengan data morfologi, analisis variasi isozim dan penjujukan DNA.

Morphological and genetic variability of Malaysian *Channa* spp based on morphometric and RAPD techniques

ABSTRACT

A morphometric and genetic study were conducted on six of the seven species that have been documented in Peninsular Malaysia; *Channa striata*, *C. gachua*, *C. marulioides*, *C. micropeltes, C. melasoma* and *C. lucius.* Eighteen side view and 11 dorsal view measurements were obtained from 81 individuals. Data was analysed by an analysis of variance, ANOVA and multivariate analyses (Principal Component Analysis- PCA and Discriminant function Analysis- DFA). Based on these statistics, generally all six species were grouped into their own species cluster with minimal overlap. *Channa gachua* was found to be the most differentiated for both analyses mainly based on size differences along the caudal peduncle (side view) and width of the head (dorsal view). Relationships among the other species were however different between the two analyses. *Channa maruloides, C. striata* and *C. melasoma* clustered very closely while *C. lucius* was a distance away. *Channa micropeltes* was isolated in terms of body depth from the dorsal to the pectoral fins along the side view. In the dorsal view *C. striata, C. lucius* with *C. micropeltes*.were very closely related while *C. melasoma* was closely related to *C. maruloides.*

Random Amplified Polymorphic DNA primers (RAPD) were used to estimate genetic variation of six *Channa* species found in Peninsular Malaysia and their phylogenetic relationship. Ten representatives for each species were analysed except for *C. melasoma* where only three individuals were obtained. Nine selected RAPD 10-mer nucleotide primers namely AB-2, AB-7, AB-14, AB-18, F2, F3, F10, F16 and F20 were used in this study. The nine primers amplified 83 scorable loci. Results showed fairly high genetic intraspecific variability among species except for *C. melasoma*. Polymorphism was highest for *C. lucius* (46.7 %) and lowest for *C. melasoma* (8.9%) although both were sympatric. Genetic distances between pairwise comparisons were

found to be in the range of 0.6 to 0.7 although a great deal of overlap was observed for between-species comparisons among all the species. The dendogram generated by RAPDistance software revealed that *C. gachua* was the most basal taxon with *C. striata* and *C. lucius* forming the terminal taxa. However, placement of species was slightly different based on the FreeTree software but the dendrogram produced had very low interspecies bootstrap support. Support for the monophyly of each species was, however, excellent at 100%.

The results demonstrated the usefulness of morphometrics in classification of species and defining shape and size differences among the *Channa* species. On the other hand RAPD can be used to assess DNA polymorphism and intraspecific variability. The applicability of RAPD analysis to systematic studies and phylogenetics should, however, be tested by comparisons with the data from morphological, isozymes variation analysis and DNA sequencing

CHAPTER 1: INTRODUCTION

1.1 The *Channa* - snakehead

The *Channa* is a group of freshwater fishes which are more popularly known as snakeheads because of its striking head resemblance to that of a snake. In fact its head profile makes it very distinct from other species. It can be found widespread in the tropics and originates from Africa, South East Asia and East Asia (Sterba, 1967; Burton & Burton, 1975; Kottelat *et al*., 1993).

1.1.1 Taxonomy and Morphology

According to Kottelat *et al.* (1993), Lee and Ng (1994), Mohsin and Ambak (1992) and Lagler *et al.* (1977), snakeheads are classified into:

Phylum	: Chordata
Superclass	: Gnathostomata
Grade	: Pisces
Subclass	: Actinopterygii
Order	: Perciformes
Suborder	: Channoidei
Family	: Channidae
Genus	: *Channa*

In Peninsular Malaysia seven species have been recognised namely *Channa striata, C. gachua, C. marulioides, C. micropeltes, C. lucius, C. melasoma* and *C. bankanensis*. They are moderate to large in size. As a group these species have some distinguishing features. Their head surfaces have large plate-like scales. The

mouths are large with sharp teeth. The fins are hyaline without spines. Dorsal and anal fins are long.

Channa striata is commercially important especially as food fish and locally known as haruan. In South East Asia it is highly regarded for its biomedical properties, believed to be beneficial in the healing of internal and external wounds due to its high content of arachidonic acid (Yaakob and Ali, 1992). *Channa micropeltes* or also known as giant snakehead, which is the largest member of this genus is now a popular delicacy in Malaysia. According to Malaysia Fisheries Statistic (DOF, 2004) aquaculture production of *C. micropeltes* was 1,162.67 tonnes for 2004. The retail value is estimated to be almost RM9.5 million. This species locally known as toman is a very popular game fish in Malaysia. Its predatory instinct combined with its ferocious nature makes it a highly exciting fish for the game fishers. Even *C. marulioides* which is considered rare is very sought after by anglers. This has led a group of anglers through their forum, Malaysian Fishing Net to suggest a 'catch and release' practice for the conservation of four *Channa* species as listed in Table 1.1.

Several members of *Channa* species are important in the Malaysian aquarium industry. In their juvenile stage *C. gachua* and juvenile *C. micropeltes* are very popular aquarium fishes; *C. gachua* due to its attractive orange/white outer fins margin and *C. micropeltes* its orange and black longitudinal stripes. However, in the case of *C. micropeltes* these attractive colours and features are lost during their growth development. It is believed that some of these fish may be illegally smuggled abroad by unscrupulous aquarium traders. However, this lucrative business sometimes poses a hazard to the environment especially in places where snakehead is not a native fish since some of the fish find their way into dams, rivers and other water bodies either accidentally or otherwise (released by traders when they mature into less attractive

and marketable product or abandoned by their owners). Having no natural predators they flourish in their new environment and become pests.

Table 1.1: Recommendations of catch and release practices for anglers regarding four *Channa* species (Malaysian Fishing Net, 2006 with some modification).

Scientific name	Local name	Status	Size Min (kg).	Size Max. (kg)	Bag Max. per day
Channa striata	Haruan	Common	0.7	1.5	2
Channa lucius	Bujok	Uncommon	0.7.	1.2	2
Channa micropeltes	Toman	Common	1.5.	3.0	1
Channa marulioides	Toman Bunga	Rare	Release	Release	Release

1.1.2 General biology of the snakeheads

Ecology

Snakeheads are very hardy animals due to the presence of several biological features that they share among their species. In particular they are air breathers possessing well developed breathing organ that are made up of vascularised pouches developed from the pharynx lying dorsal to the gill chamber (Soong, 1949; Olson *et al*., 1994) but they still retain their gills for both respiration and non-respiration purposes. *Channa striata* is very dependent on its breathing organ; they will drown if they cannot come up for air. Having this apparatus enable them to withstand extreme condition of low dissolved oxygen and dry season. It also allows them to move from one water body to another across semi-watery areas by the undulation of their body. Another biological advantage they possess which assist them in surviving extreme conditions lies in the structure and chemical component of the subcuticle namely in the epidermis and dermis as exemplified in *C. striata,* (Wee, 1982). The dermis consists of many mitochondrial cells which account for its high rate of metabolism. Mucous cells are densely packed in the skin layer to keep the skin moist and to reduce water loss through the skin (Wee, 1982).

Feeding

In nature snakeheads are carnivorous which feed only on live animals. They feed on almost anything including small fishes, insects, crustaceans, frogs and even baby turtles (Lee and Ng, 1994). They are a threat to prawn and fish culture if and when they manage to invade these cultured ponds. In the aquarium, they have to be kept with fish of the same or bigger sizes; smaller fishes inevitably end up as their next

meal. They even prey on members of their own species or even their young. However, this cannibalistic nature is sometimes beneficial to the aquaculturists in controlling the density of their culture and keeping uninvited guests at bay (Wee, 1982).

Reproduction

The *Channa* are nest brooding species. Nests are built by the mating parents for nesting the fertilized eggs, in a shallow area by biting off vegetation. The fertilized eggs are yellowish in colour forming a thin film at the water surface. The offspring is jealously guarded by one or both parents. They are territorial and some species have been known to attack people who venture into their territory. Most of the snakehead species are monogamous. *C. gachua* is a mouth brooder. Usually snakehead juveniles can be found swimming in schools while the adults swim alone or in pairs (Kottelat *et al*., 1993).

According to Yaakob and Ali (1992), mating in *C. striata* begins with the male courting its partner by encircling and nudging it. During actual spawning the male will position itself to fertilise the eggs as they are released. According to Parameswaran and Murugesan (1976) this mating behaviour will last from 15 to 45 minutes. The colours of the fertilized eggs are golden yellow to yellowish brown with a size ranging from 1.15 to 1.46 mm and containing a high amount of lipids that allow them to float to the surface. The eggs hatch 24 to 48 hours post fertilization.

1.1.3 Morphological taxonomy of the snakeheads

The systematic of the six snakehead species investigated in this study is described below. Descriptions are based on Kottelat *et al*. (1993) and Lee and Ng (1994).

The dorsal, anal and caudal fin margins of *C. gachua* (Plate 1.1) are usually reddish. It has a dark line across the body which lightens in intensity as it gets older. There are 3 to 3½ scales between the lateral line and base of anterior dorsal rays. This species can be found in hill streams in Sundaland (Peninsular Malaysia, South of Isthmus of Kra, Sumatra, Borneo and Java), Bali, Indochina, India and Sri Lanka.

The upper profile of the *C. lucius* (Plate 1.2) head is concave usually with a series of large dark blotches at the sides and oblique bars on the belly. Its vomer and palatines have a single row of canines between which smaller teeth are inserted. The scales between the lateral line and base of the anterior dorsal rays are 5½. It can be found in streams in forested areas in Sundaland and Indochina.

Black ocellus with white edge dominates the upper part of the caudal base of *C. marulioides* (Plate 1.3). It does not have canines on either the vomer or palatine. There are 3½ scales between the lateral line and anterior rays of dorsal. It can be found in Sumatra, Bangka, Belitung, Borneo and Malaysia.

Channa melasoma has pectoral fins that can be as long as the distance rear of the eye to edge of opercle (Plate 1.4). It does not have canines on either the vomer or palatine. There are 4½ scales between the lateral line and base of anterior dorsal rays. It can be found in Sumatra, Malaysia including Borneo, Indonesia and the Philippines (Palawan).

Plate 1.1: *C. gachua* (pic. by Amiruddin Ahmad)

Plate 1.2: *C. lucius* (Copyright: www.jjphoto.dk)

Plate 1.3: *C. marulioides*

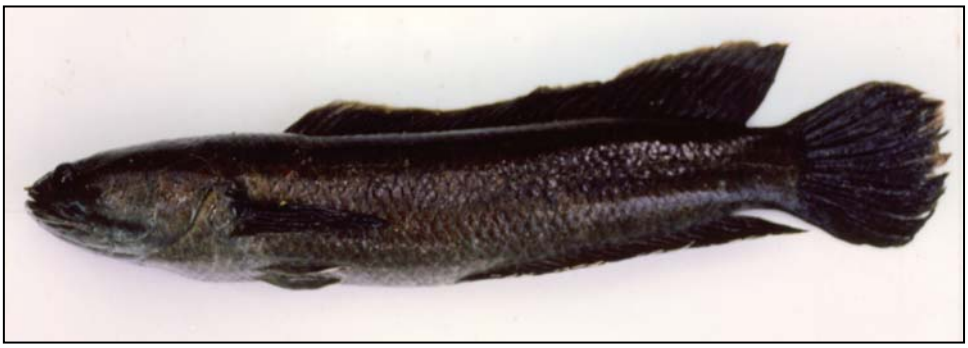

Plate 1.4: *C. melasoma* (pic. by Amiruddin Ahmad)

Juvenile of *C. micropeltes* (Plate 1.5) can be recognized by their two black and an orange stripe from snout to caudal. These stripes break up into irregular spots and eventually fade in older individuals. Juveniles can be found moving in schools while the adults are usually solitary and aggressive. It has a vomer and palatine with a row of small teeth and a row of strong canines. This snakehead species can be found in Sundaland, Indochina and India.

Juvenile *C. striata* (Plate 1.6) is bright orange with two light brown dorsal lateral stripes. The ventral surface is white from head to belly but brown along the anal fin. Orange colour starts to disappear when it gets older. The sides of the body have chevron-shaped bars pointing forward. This striation run diagonally along both sides of the body but the upper part of it is usually indistinct in older specimens. It does not have canines on the vomer and palatines. There are 4 to 5 scales between the lateral line and base of anterior dorsal rays. This is the most widely distributed snakehead species ranging from Sundaland (Peninsular Malaysia, South of Isthmus of Kra, Sumatra, Borneo and Java), Sulawesi, Lesser Sundas, Molluccas, India, Indochina and China although in some of these places it is an introduced species. This species is also the most widely cultured for food fish and economically is very important.

Plate 1.5: *C. micropeltes* (pic. by Amiruddin Ahmad)

Plate 1.6: *C. striata*

1.2 Introduction on morphometrics

The word morphometrics is derived from two Greek words: "morph," meaning shape or form, and "metron", meaning measurement. Generally, it is a method of extracting measurements from shapes and in most cases was applied to biological topics in the widest sense. Daly (1985) defined morphometrics as a measurement and quantitative analysis for morphology or shapes. Basically morphometric is a method that involves quantitative analysis of size measurement, shape and other morphological characters. Theoretically, this systematic characterization of a fish form will increase the likelihood of extracting differences with biological meaning within and between species. Conventional method of collecting morphometric data does not concern with allometry (growth of different body parts) or its variations among populations or growth stages (Strauss and Bookstein, 1982). It was used to capture and summarise information on shape variation by sampling shape in an unplanned kind of way that has not necessarily any connection between each measurement of the objects being analysed.

The use of morphometrics has gained wide acceptance in the contemporary biological scene. As such it is increasingly used as a necessary complement to molecular studies due its low budget requirement and acceptable resolving power of discrimination. Among others it can be used to quantify patterns of growth and development both ontogentically (development history of individual) and phylogenetically (sequence of changes occurred during evolution of species or group of organism) (Baumgartner *et al.*, 1988; Lee & Ng, 1994) as well as magnitudes of variation within and among species and measure their causal effects (Ruiz-Campos *et al.*, 2003). In the phylogenetic contexts, it has been utilised in finding new and sharpening the definition of old character states (Elewa, 2004).

According to Cadrin (2000), the wide use of multivariate methods for stock identification was initiated by the work of Royce (1957) and since then applications of

multivariate morphometrics flourished. Saila and Flowers (1969) provided an early example of using discriminant analysis to study geographic variation by analysing the morphometrics of the American lobster. The advent of electronic computers, digitizers coupled with more rigorous statistical analyses and other peripherals over the past four decades further facilitated its utilisation in fishery stock identification and conservation (Cadrin and Friedland, 1999; Cadrin and Friedland, 1999; Kassam *et al*., 2004).

The introduction of the truss network measurements in place of traditional measurement has brought about greater insights into the biological interpretation of size and shape differences. The truss network measurements have been defined as a series of measurements between landmarks that form a regular pattern of contiguous quadrilaterals or cells across the body form (Winans, 1984). Landmark characters are coordinate based characters; the organisms may be studied and compared through the use of appropriate methods for describing differences in shape and size (Corti and Crosetti, 1996). Furthermore, carefully selected common features among specimens also known as homologous landmarks are more effective for describing shape than arbitrarily located landmarks (Bookstein, 1990). For example, Winans (1987) found that homologous landmarks among finfish, such as the attachment point of fin membranes, were more effective for group discrimination than landmarks located on extremities, such as the narrowest portion of the caudal peduncle. In addition defining the location of homologous landmarks is also generally more repeatable among different researchers than locating landmarks that are based on extremities (Cadrin, 2000). Several researchers have compared performances of traditionally measured finfish dimensions to box-truss distances and found that the latter provided a more accurate classification of individuals (Strauss and Bookstein, 1982; Winans, 1987; Schweigert, 1990; Roby *et al*., 1991). The assessment of intra and inter population variation, could also determine how and to what extent differences among individuals are shaped into the differences that separate races and species (Williq *et al.*, 1986).

Some of the advantages of using a truss network as described by Strauss and Bookstein, (1982) include;

1. systematic coverage across the form; in contrast to traditional characters which provide highly uneven coverage thus enabling reconstruction of the original configuration of landmarks.
2. the application of rigorous statistical analyses such as Analysis of Variance (ANOVA) and multivariate analysis (PCA and DFA) which could be correlated to differentiation among taxonomic groups and geometrical interpretations of studied organisms.

However as they are polygenically inherited, heritability is low and these characters may be subject to considerable environmental plasticity (Karakousis *et al*., 1991). As mentioned morphometric data is analysed using univariate and multivariate analysis. In univariate analysis, multiple comparison tests such as Student Newman Keuls (SNK) is often used to test significance of mean between groups (Sokal & Rohlf, 1981). Therefore a series of univariate ANOVAs actually test a series of null hypotheses concerning equalities of means for each variable independently (Williq *et al.*, 1986). However, multivariate analysis of a set of phenotypic characters is regarded as a more appropriate method than the use of single characters for determining the extent of genetic diversity (Karakousis *et al.*, 1991). For multivariate analyses some of the frequently statistical technique employed include discriminant analysis (DFA), principal component (PCA) and coordinate analysis. Many studies have documented its utilization as an efficient tool for various biological investigations such as in stock identification in management programs and fisheries (Winans, 1984; Barriga-Sosa *et al*., 2004) investigating taxonomic problems (Doadrio *et al.,* 2002) and population differentiation (Costa-Pierce, 2003).

1.2.1 Principal Component Analysis (PCA)

Principal component analysis (PCA) is used to describe body shape independently of body size. This is a concise statistical method for investigating patterns of variance in multivariate data by deriving a few uncorrelated indices based on objective combinations of correlated variables (Cadrin, 2000). The first principal component (PC1) is derived as a weighted combination of all log measurements according to patterns of correlation among morphometric variables. PC1 was generally positively correlated with all linear dimensions of size. Teissier (1960) related this pattern to the simple observation that all dimensions increased during growth. Thus, the PC1 score of each specimen (a weighted combination of all morphometric variables) was interpreted as an index of general size. Jolicoeur (1963) modified Teissier's approach using a PCA of the variance-covariance matrix rather than a correlation matrix to study multivariate allometry. Teissier (1960) interpreted the second principal component (PC2) as a "shape component." The effect of general size was accounted for by PC1, and most of the residual variance caused by shape differences was resolved by PC2. Unlike PC1, which was positively correlated to all dimensions, PC2 was positively correlated to some variables, and negatively correlated with others, as the latter reflects divergence in growth trajectories among allometric features (Shea, 1985; Cadrin, 2000).

PCA was designed to explore variance within one homogeneous sample eventhough pooled-group PCA is a useful exploratory method, PCA of heterogeneous groups characterizes multimodal distributions with conventional means, variances, and covariances. Within- and among-group variability is mystified in pooled-group PCA (Reyment, 1985; Thorpe, 1988; Marcus, 1990).

PCA has been used to view changes in character covariability during early growth and shape development (Atchley and Byrant, 1975; Green, 1976; Winans, 1984). Teissier (1938) applied PCA to eight log (base 10) transformed dimensions of spider crab, pioneering the use of multivariate techniques for morphometric research (Blackith and Reyment, 1971; Sundberg, 1989; Reyment, 1990).

Study on sympatric cichlids from Nicaraguan lake by Barluenga *et al.* (2006) showed that PCA based on nine landmarks (left) shows a clear separation in body shape between *Amphilophus citrinellus* and *A. zaliosus* with little overlap while another PCA based on ten landmarks showed clear differences in this structure between the two species

1.2.2 Discriminant Function Analysis (DFA)

An alternative method for multi-group analysis of many morphometric variables is discriminant analysis. Sir Ronald Fisher introduced discriminant analysis as a multivariate extension of analysis variance (ANOVA) to study variation and covariation among taxonomic groups (Fisher, 1936). Discriminant analysis tests significance of group differences by deriving a weighted combination of variables which maximizes differences among groups. As in ANOVA, total sums of squares (and cross products) are partitioned into within- and among-group portions. Similar to PCA, which transforms data to optimise the percentage of variance explained by successive components, discriminant analysis produces an ordination variable that has maximum differences among groups. Unlike PCA, which emulates a multivariate allometric equation, discriminant analysis is not as biologically interpretable. Discriminating power of variables can only be approximated indirectly using univariate F ratios, entry order of stepwise analyses, standardized discriminant coefficients, or pooled within

groups correlation to discriminant scores (Marcus, 1990). Allometric interpretations can only be made indirectly by comparing coefficients from PCA and discriminant analysis (Cadrin, 1995). A discriminant analysis derived from specimens with known group membership (*i.e.* intrinsic data) can be used to classify other specimens with unknown membership. Furthermore, classification of extrinsic specimens with known membership could be confirmed.

1.2.3 Studies based on the truss network

Discriminant function analysis (Atchley and Byrant, 1975; Green, 1976; Winans, 1984) has been widely utilized in study on many organisms including fishes (Barriga-Sosa *et al.*, 2004; Doherty and McCarthy, 2004; Erguden and Turan, 2005). Doherty and McCarthy (2004) utilized DFA to the transformed morphometric data of Irish Arctic char *Salvelinus alpinus* to classify the fish into discrete groups and calculate the overlapping percentage between each group based on Mahalanobis squared distance. They were able to achieve their objectives based on this analysis.

A study by Erguden and Turan (2005) on genetic and morphologic structure of sea bass *Dicentrarchus labrax* populations in its distributional ranges in Turkish coastal waters also found that DFA was useful in assigning individuals into their original population. It successfully classified populations of the Aegean Sea (100%), Black Sea (97%), Mediterranean (83%) and Marmara (70%) to their original populations.

Baumgartner *et al.* (1988) successfully defined *Gasterosteus aculeatus* (threespine stickleback fish) species complex based on body form differences using truss configuration. They found that these two species which occurred sympatrically in the

Enos Lake had morphometrically diverged about 13 000 years ago, since the deglaciation of Vancouver Island.

Parsons *et al.* (2003) compared several traditional morphometric analyses including truss against a newer geometric analysis involving thin-plate splines applied to a common data set of morphologically variable new world cichlids *Amphilophus citrinellus* and *A. zaliosus*. They found that prior morphometric studies using truss-based measures could be reliable despite the claim that newer geometric morphometric methods can better estimate shape because the latter analyse the geometry among the locations of all landmarks simultaneously rather than the linear distances between pairs of landmarks. They also suggested that traditional morphometric methods were also perhaps more flexible than geometric methods because truss lengths, lengths of gill rakers and sizes of internal organs can more easily be combined in a single analysis.

Ruiz-Campos *et al.* (2003) used 25 standardized morphometric characters following a box truss protocol to analyse morphometric variation of 17 wild trout populations of the genus *Oncorhynchus* from northwestern Mexico. Based on sixteen linear characters they were able to distinguish the three taxa examined. They were also able to differentiate two distinctive groups of populations based on the squared Mahalanobis' distances.

Poulet *et al.* (2004) used morphometric and meristic features, otolith shape descriptors and protein electrophoresis in order to assess whether the Rhône River delta waterbodies houses one or several pikeperch populations. They found that all characters except the meristic counts highlighted the existence of two subpopulations: one in the drainage network and one in the irrigation network. This meant that the truss network method was more efficient than meristics in discriminating between the

subpopulations. Their work also signified the relevance of morphometric and genetic analysis.

In recent years the combination of morphometric and genetic data have been frequently utilised by various researchers in population studies. Hedgecock *et al.* (1988) used electrophoretic and morphometric approach to confirm the status of three sympatric species of thread herring. Erguden and Turan (2005) investigated genetic and morphological structure of sea bass, *Dicentrarchus labrax* populations throughout its distributional ranges in Turkish coastal waters. They found that there were no genetic differences between the Black Sea and Mediterranean populations using 9 loci while morphometric study has correctly classified four different populations.

1.3 Genetic in Fisheries and Aquaculture

Genetics analysis has much to offer fisheries managers, especially in the provision of tools enabling unequivocal specimen identification and assessment of stock structure (Ward, 2000). Genetic data have become increasingly important in assessing the gene flow between populations, which is crucially important for the maintenance of genetic diversity. Genetic technologies have been recognised for their usefulness in species identification (Palumbi and Cipriano, 1998; Prioli *et al*., 2002), monitoring fisheries (Menezes *et al*., 2006), aquaculture (Liu *et al*., 1998; Barriga-Sosa *et al*., 2004) and enhancement operations (Marzano *et al*., 2003). Genetic data has also been very useful in setting up conservation priorities (Dizon *et al*., 1992; Bernatchez *et al.*, 1995). In many situations, genetics may be the best way to decide whether a species is worthy of special protection under Endangered Species Act or other forms of management. It allows calculated decisions on the course of action to be taken for the protection and conservation (Leuzzi *et al*., 2004; Mataso *et al*., 2004) and managing

different stocks (Salini *et al*., 2006). Genetic uniformity of a threatened species will open the option of restocking by translocation (Teske *et al*., 2003). On the contrary highly differentiated populations would mean that each population must be independently managed. Genetic variability of depleted population may be enhanced through replenishment with appropriate stocks by the use of carefully planned programs based upon knowledge of life history, quantitative genetics and DNA variation (Brown and Epifanio, 2003). Generally, individuals with greater genetic variability have higher growth rates, developmental stability, viability, fecundity and resistance to environmental stress and diseases (Carvalho, 1993; Dinesh *et al.*, 1996).

Similarly, knowledge on the genetic structure and variability of stocks should be a prerequisite of any aquaculture program. In aquaculture practices improper management of broodstock or limited number of broodstock could lead to loss of genetic variation or inbreeding. This can happen because potential mates are more likely to be closely related. Proper management of genetic data using genetic tools can be used to avoid inbreeding and to improve the quality of fish produced (Islam and Alam, 2004). Wasko *et al.* (2004) used genetic study to analyze and evaluate the levels of genetic diversity of Amazonian matricha, an important neotropical freshwater fish. Their study of wild stock and three captive stocks showed that the captive stocks had lower genetic variation compared to the wild stock. However, they also found that one of the captive stocks had higher level of polymorphism compared to the other stocks. They attribute this high level of polymorphism to the choice of genetically non related breeders or application of different reproductive strategies.

1.3.1 Molecular techniques

In recent years, a wide range of new molecular techniques have been explored and reported for fishes and shellfishes (Lehmann *et al*., 2000; Jayasankar, 2004; Jin *et al.,* 2006). Several DNA techniques for evaluating genetic variability in fish species are available and are widely used (Harris *et al*., 1991; Mjolnerod *et al*., 1997; Coughlan *et al*., 1998; Norris *et al*., 1999; Wasko and Galetti, 2002; Barman *et al.,* 2003; Hassanien *et al.,* 2004; Matoso *et al.,* 2004; Jayasankar, 2004; Jin *et al.,* 2006). Molecular techniques can be categorized into two main categories namely protein and DNA markers. A brief introduction on these two types of markers is given below.

Protein Pattern

Allozyme electrophoresis is a diagnostic method that examines variation among that class of proteins which controls a large amount of cell metabolism. Isozymes refer to all of the different forms of an enzyme that perform the same catalytic function while allozymes refer to only those genetically different forms of an enzyme that are encoded at the same locus (May, 2003). This technique became available in mid-1960s and is inexpensive, requires simple equipment and large quantity of data can be produced quickly. Many baseline data are available. It has also been proven to be informative for monitoring inheritance of a single gene and can also be used to build linkage maps (May and Johnson, 1993; Morizot *et al*., 1991) or to study evolution by genomic duplication exhibited by some fish families (Allendofr and Thorgaard, 1984). Numerous studies have reported differences in protein allele frequencies between stocks of fishes (Ward and Grewe, 1995, Tudela, 1999). This type of data offer great potential for fisheries management such as in the study on the connectivity of different coral reef populations in the Western Indian Ocean (Ridgway, 2005).

Study on the genetic structure of European anchovy by Tudela (1999) using enzyme electrophoresis showed that no apparent genetic structure was detected in an area covering 1000 km of open coast off southern Africa. They suggested more precise and sensitive genetic techniques were required to validate their findings. A study by Salini *et al.* (2006) on two species of red snappers, *Lutjanus malabaricus* and *L. erytropterus* using both allozymes and mtDNA techniques found that allozyme data suggested the presence of seven discrete populations while mtDNA only discriminated two populations. According to them, one of the possible explanations for observed differences in population discrimination by allozymes and mtDNA may be due to insufficient gene sampling in the mtDNA control region. Thus more rigorous analyses of mtDNA by targeting more restriction sites could have uncovered more differences.

Although many enzymes have been shown to exhibit high levels of allozymic variation, protein genes represent only about 10% of the genome and the results obtained using this method do not represent the whole genome. Newer molecular techniques utilizing DNA studies which offer advantages over allozymes data are now often utilised to complement allozymes data (when available).

DNA Markers

More recently, genetic variation at molecular level has been used in population genetic studies using a variety of technical approaches. DNA markers can be divided into two; (1) Mitochondrial DNA (2) Nuclear DNA markers.

Mitochondrial DNA markers

Mitochondrial DNA makes up less than 1% of eukaryotic cells DNA and is located within organelles in the cytoplasm (Gray, 1992). It encodes genes involved in the

central metabolic processes. Mitochondrial DNA is maternally inherited (Avise *et al*., 1992) which means that all offspring regardless of sex will inherit their mothers' haploid genotype. This gives it added advantage over nuclear DNA since it evades recombination unlike nuclear DNA. Mitochondrial DNA is very popular for population genetic studies and examination of phylogenetic relationship among closely related species (Matthews *et al*., 2002, Mayden, 2002; McCafferty *et al*., 2002).

Mitochondrial has been widely utilized in fisheries which include study of variations among individuals in one species (Kai *et al.,* 2002; Norazila and Patimah, 2002; Degani, 2004), examination of variation within and among populations for the study of different fish stocks for management practices (Sullivan *et al.,* 2004; Menezes *et al*., 2006), phylogenetic study of variation among species and genus (Mayden, 2002; Billington, 2003). It also provides much needed information for conservation and rehabilitation of fish species such as study by Bardakci *et al.* (2006).

Mitochondrial is being utilized to investigate the outcome of restocking programme of brown trout using domesticated strains of *Salmo (trutta) trutta* (Marzano *et al.,* 2003*).* This domestic stock has been previously transferred to most of the water bodies in Italy. The repopulation was carried out without considering the existence of autochthonous populations of *S. (trutta) macrostigma*. They found that existence of *S. (trutta) macrostigma* was influenced by restocking policies. The presence of the *S. (trutta) macrostigma* seemed quite patchy in area where the restocking is practiced. The restocking program should consider the importance of genetic in managing the fisheries.

Nuclear DNA

A wide spectrum of nuclear DNA technology such as RAPD, AFLP and microsatellite has been applied to address various fisheries issues such as stock identification, mixed stock analysis (Hanfling *et al*., 2005)., conservation, domestication and taxonomy (Callejas and Ochando, 1998, Prioli *et al.*, 2002).

Polymerase Chain Reaction (PCR)

Development of polymerase chain reaction for DNA amplification represents one of the most important molecular techniques in the last decade. PCR technique has several advantages over traditional, *in vivo* method of cloning DNA. It enables genetic investigation using only a small amount of DNA. Basically PCR is a 3-step chemical amplification process consisting of melting, annealing and extension of DNA molecule. The basic components needed in every PCR reaction are one or more molecule of target DNA, oligonucleotide primer, free nucleotide (dNTP) and a thermo stable DNA polymerase in a suitable reaction buffer. The amplification mixture is first heated to break the hydrogen bonds and separate the double-stranded DNA. Then, the melted DNA is cooled to allow annealing of primer that acts as initiation templates for replication by the DNA polymerase with the temperature raised slightly higher. These processes will be repeated for about 30 to 40 cycles. In each cycle, newly synthesized DNA strands become templates for the next replication thus producing over a million fold of products. Theoretically each amplification cycles will double the amount of target DNA after every cycle which results in exponential increase in PCR product. PCR products can be visualised on an agarose gel using ethidium bromide staining (Brown and Epifanio, 2003).

Random Amplified Polymorphic DNA (RAPD)

The random amplified polymorphic DNA (RAPD) technique was first introduced by Williams *et al.* (1990). It is a technique based on the PCR amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsh and McClelland, 1990; Williams *et al.*, 1990). The random amplified polymorphic DNA (RAPD) technique utilises single, arbitrary, decamer DNA oligonucleotide primers to amplify regions of genome based on the polymerase chain reaction Hadrys *et al*., 1992; Williams *et al*., 1993). Being a PCR based method, it utilizes only a small quantity of DNA template (usually less than 50 ng), one or more oligonucleotide primer, free nucleotides and *Taq* polymerase in a suitable reaction buffer. Oligonucleotide primer used must have high GC content to enable amplification. The primers used are about 10 bases in length and is capable of binding to and amplifying complimentary sites of the specific region of the genome (Williams *et al.*, 1990). Priming sites are abundant and distributed randomly across the genome. Polymorphism in these regions among various genomic DNA is represented as different banding patterns. These markers are inherited in a Mendelian fashion (Liu and Cordes, 2004).

Characters assessed through RAPD are useful for genetic studies because they provide various types of data- taxonomic population, inheritance pattern of various organisms including fishes (Brown and Epifanio, 2003, Degani, 2004; David and Pandian, 2006). This technique is inexpensive and rapid compared to other genetic techniques as development of markers and prior sequence of target DNA is not needed. Universal sets of oligoprimers are commercially available. As the amount of DNA requirement is very small it can be utilized for studies with limited genomic DNA such as museum specimens and rare samples. RAPD directly examines the genomic DNA compared to protein polymorphism assay which acts as predictor of overall levels