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Genetic Variation of Snakehead Fish (*Channa striata*) Populations Using Random Amplified Polymorphic DNA

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Abstract: The objectives of this research were focused on the genetic relationships among four populations of *Channa striata* distributed in Peninsular Malaysia. A set of OPA primer with 10-mer was used to assay polymorphisms among populations. Different RAPD fragment pattern were observed for different locations. A total of 8 primers produced 42 polymorphic bands among all populations. Using this technique, only slight differences in genetic diversity were detected among locations of *Channa striata* within Peninsular Malaysia although the diversity of all locations analyzed together differed from the reference populations. Differences in genetic distance between populations may be due to selection pressure of pollutants on fish. These results are discussed in relation to implications of RAPD assays in the evaluation of genetic diversity.

Key words: Snakehead fish, *Channa striata*, random amplified polymorphic DNA, RAPD, fingerprinting, primer, population, genetic diversity

INTRODUCTION

Snakeheads or Ikan Haruan are fish belonging to the family Channidae. They are also known as murrels and serpent-headed fish. At the present, there are 30 snakehead species found in the world including 3 species in Africa and the remains in Asia. They distribute naturally in rivers, canals, lakes, swamps, marshes, earthen ponds and rice-fields. They are the important food fish species in these areas, particular in Malaysia. Other than that, snakeheads are important in pharmaceutical products and traditional medicine.

Although DNA fingerprinting offers great potential in aquaculture and in fisheries as a tool for identification of individuals (Dinesh *et al*., 1993; Bardakci and Skibinski, 1994; Takagi and Taniguchi, 1995; Dinesh *et al*., 1996, Meruane *et al*., 1997; Nei and Li, 1979; Heist and Gold, 1999; Jong-Man, 2001) and population genetic (Hallerman and Beckmann, 1988; D'Amato and Corach, 1996; Bielawski and Pumo, 1997; Smith *et al*., 1997; Mamuris *et al*., 1998). The RAPD analysis is also employed in differentiating sex chromosome (Iturra *et al*., 1998), genetic inheritance (Elo *et al*., 1997), gene mapping (Liu *et al*., 1999) and fish conservation (Fritzch and Rieseberg, 1996; Dioh *et al*., 1977).

Using RAPD fingerprinting on fish has been limited so in the current study, this technique was applied to analyze the genetic relationships among snakehead populations. The objectives of this experiment were focused on detection of RAPD pattern for snakehead fish and determination of the genetic variation among snakehead populations.

MATERIALS AND METHODS

Sample preparation: Samples from 56 individuals of four populations of *Channa striata* in Peninsular Malaysia were collected in 2002. Flesh was preserved in 95% ethanol.

Quantification of DNA samples: Genomic was extracted using Phenol-Chloroform method (Taggart *et al*., 1992). Genomic quantity was measured using UV spectrophotometer (UV-VIS KONTRON). DNA sample was filled up in capillary tube. Quantity of DNA was represented by absorbance reading at 260 nm and purity of DNA was estimated by ratio of absorbance reading between 260 and 280 nm.

Primers: Twenty primers (OPERON) were used to amplify genomic DNA. Operon 10-mer kits contained 10 base oligonucleotide primer (Table 1) using in genetic mapping.

Amplification of DNA: Samples were amplified in 25 μL reaction mixture containing 1X PCR buffer (Biotools), 5 mM $MgCl_2$, 5 pmoles primer, 0.2 mM dNTP (Biotools),

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Table 1: The sequence of 20 primers using for RAPD analysis

Name of primer	Nucleotide length	Sequence (5'-3')	G+C%	Molecular weight	`Picomoles
OPA-01	10 mer	CAGGCCCTTC	70	2955	6014
OPA-02	10 mer	TGCCGAGCTG	70	3035	5493
OPA-03	10 mer	AGTCAGCCAC	70	2988	5192
OPA-04	10 mer	AATCGGGCTG	60	3059	5088
OPA-05	10 mer	AGGGGTCTTG	60	3090	5192
OPA-06	10 mer	GGTCCCTGAC	70	2995	5742
OPA-07	10 mer	GAAACGGGTG	60	3108	4625
OPA-08	10 mer	GTGACGTAGG	60	3099	4892
OPA-09	10 mer	GGGTAACGCC	70	3044	5158
OPA-10	10 mer	GTGATCGCAG	60	3059	5088
OPA-11	10 mer	CAATCGCCGT	60	2979	5531
OPA-12	10 mer	TCGGCGATAG	60	3059	5088
OPA-13	10 mer	CAGCACCCAC	70	2933	5493
OPA-14	10 mer	TCTGTGCTGG	60	3041	5783
OPA-15	10 mer	TTCCGAACCC	60	2939	5783
OPA-16	10 mer	AGCCAGCGAA	60	3037	4710
OPA-17	10 mer	GACCGCTTGT	60	3010	5654
OPA-18	10 mer	AGGTGACCGT	60	3059	5088
OPA-19	10 mer	CAAACGTCGG	60	3028	4988
OPA-20	10 mer	GTTGCGATCC	60	3010	5654

Where, A: Adenine T: Thymine G: Guanine C: Cytosine

1 unit of Taq polymerase (Biotools) and 25 ng of genomic DNA. PCR amplification was conducted following Dinesh *et al.* (1993) with some modifications. Amplification was run using Perkin Elmer Cetus Gene Amp PCR System 2400 programmed for 45 cycles of 5 min. denaturation at 94°C, 1 min low stringency annealing at 55°C and 1.5 min primer extension at 72°C. At the end, a final extension for 5 min was performed at 72°C.

PCR products were run in horizontal electrophoresis tank system. Ten microliter of product mixed with 3 μL dye was loaded in 1.7% agarose gel and run electrophoresis at 55 V in 1X TBE buffer. The gel was stained with ethidium bromide for 1 h and washed in distilled water for 30 min. Then the gel was viewed under ultraviolet light using Image Master VDS (Pharmacia Biotech).

Data analysis: Data was analyzed using RAPDistance Package Software version 1.04 (Amstrong *et al.*, 2004). The presence or absence bands were recorded on photograph. The bright band was scored as present (1) and no band was as absence (0). Then data was input into data analysis package.

Genetic similarity indices (SI) were calculated using the method of Lynch (1990). The formula for SI is given as

$$SI = 2n_{xy}/(n_x+n_y)$$

with: n_{xy}, the number of fragments shared by individuals x and y.
n_x and N_y, number of fragments scored for each individual.

Genetic distance-UPGMA dendrogram. The scored data were used to generate the dendrogram with RAPDistance computer software, using the Neighbor-Joining Tree Program to produce the desired tree or dendrogram of cluster analysis using Similarity Index of Dice (Lynch, 1990).

$$S_{ij} = 1 + S'_{ij} - 0.5 (S_i + S_j)$$

Where, S_i and S_j were the value of S for population I and j, respectively and S'_{ij} was the average similarity between random paired individuals from populations I and j. It was possible for the value of S_{ij} to exceed 1.

S'_{ij} was also converted to a measure of genetic distance (D_{ij}) using the following equation:

$$D'_{ij} = -\ln [S'_{ij} / (\sqrt{S_i S_j}] \text{ (Lynch, 1991)}$$

D'_{ij} values were used to construct dendogram using the unweighed pair-group method of analysis (UPGMA) (Sneath and Sokal, 1973).

RESULTS

Genomic DNA: A total of 56 samples from four populations was extracted using Phenol-Chloroform method. The purity of genomic concentration varied from 35.69 to 71.79 μg μL^{-1} (ratio A_{260}/A_{280}). The quality of DNA extracted was represented by running electrophoresis on 0.8% agarose gel. The consistently good quality of high molecular weigh DNA was obtained using the Phenol-Chloroform technique for a routine total DNA isolation from 12 species of the genus of *Salmo*, *Salvelinus*, *Oncorhynchus*, *Coregonus* and *Thymalus* (Taggart *et al.*, 1992). This method was also applied isolating genomic on the mudskipper species, *Perophthalmus schosseri*, given more quantity and quality of fish DNA than other kits available in the market

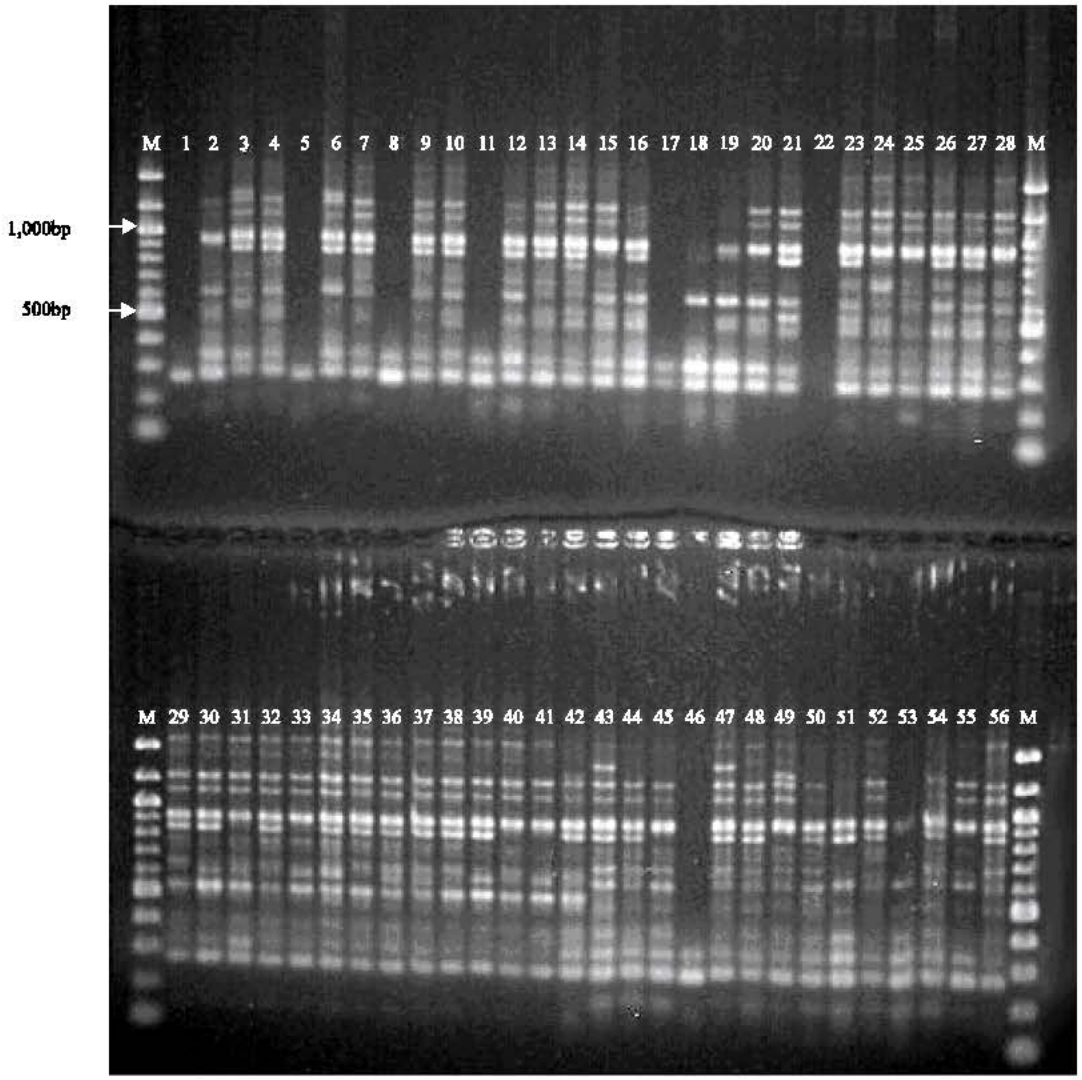

Fig. 1: RAPD patterns obtained from *Channa striata* population using primer OPA 11. Lane M: 100 bp DNA ladder (Biolabs), lane 1-14: Johore, 15-28: Perak, 29-42: Terengganu, and 43-56: Perlis

(Shima, 1999). The reports of Kim (1998) on river catfish (*Mystus numerus*) and Asma (1999) on Tiger barb (*Puntius tetrazona*) that Phenol-Chloroform technique gave good quality and high quantity of genomic of fish.

PCR optimisation.: Different *Taq* polymerases amplified different DNA banding. Saiki (1992) found that the optimum concentration of *Taq* polymerase varied from 1 to 4 units per 100 μL reaction. Increasing the amount of enzyme could result in greater production of non-specific PCR products and reduced of the desired target fragment. Similarly, the current study found that different concentrations of *Taq* polymerase gave different banding pattern. The concentration of *Taq* polymerease of 4 U μL^{-1} gave the best banding patterns.

The deoxynucleotique triphosphate (dNTP) are usually present at 50 to 200 μL of each (dATP, dCTP, dGTP, and dTTP). If higher concentration may tend to promote minicorporation by the polymerase (Saiki, 1992). In present study, different concentration of dNTP amplified different banding pattern. The concentration of dNTP at 0.5 mM and gave the best RAPD patterns.

The concentration of magnesium chloride ($MgCl_2$) effects on specific and yield of amplification. Concentration of about 1.5 mM is usually optimal (with 200 μL each dNTP). If excess Mg^{2+} is resulted in the accumulation of non-specific amplification products and insufficient Mg^{2+} can reduce the yield (Saiki, 1992). In this study, the different banding patterns were resulted in various concentration of magnesium chloride. The

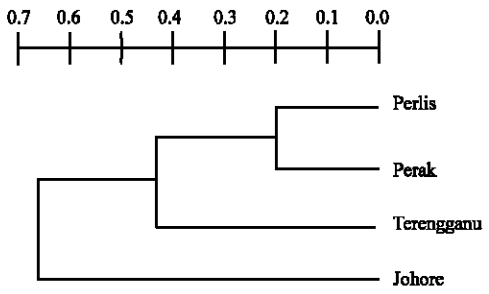

Fig. 2: UPGMA dendrogram of population snakeheads, *Channa striata* based on values of genetic distance (D'_{ij}) calculated from data for all 8 primers

concentration of magnesium chloride at 1.5 mM gave the best banding patterns.

Twenty OPA primers were tested after optimum reaction condition. Then the primers having over 3 bands were used running PCR. In the current study, OPA-01, OPA-06, OPA-08, OPA-09, OPA-11, OPA-12, OPA-15 and OPA-20 were amplified RAPD patterns.

RAPD polymorphism among snakehead species: A total of 8 random primers (OPA-01, OPA-06, OPA-08, OPA-09, OPA-11, OPA-12, OPA-15 and OPA-20), 60-70% of G+C rich sequences were screened for DNA amplification using template DNA from 4 populations. All of them were able to generate reproducible amplification products with the 8 primers tested. Primers with different oligonucleotide sequence generated amplification fragment different in number and size. The number and size of fragments amplified by these primers varied from 1-12 bands and 250-1500 bp.

RAPD profile of *Channa* population: Analysis of PCR product by gel electrophoresis showed that different primers and various populations gave different numbers of band. Every primer gave 2-8 bands in OPA-01, OPA-12, 3-10 bands in OPA-06, OPA-20, 4-7 bands in OPA-08, 3-12 bands in OPA-09, OPA-15, and 1-12 bands in OPA-11. The bands amplified using OPA-11 is presented in Fig. 1.

RAPD analysis is useful since different number and size of fragments in different fish species can be obtained. Different fish species were given different number and size of bands such as orange roughly (Smith *et al*., 1997), striped bass (Bielawski and Pumo, 1997), *Salmo* sp. (Elo *et al*., 1997), channel catfish (Liu *et al*., 1999) and tiger barb (Asma, 1999).

The genomic DNAs from different locations of snakehead were extracted using Phenol-Chloroform method gave high concentrations. The variation in numbers of amplified bands was observed with drastic changes. Higher DNA template concentration led to amplification of more bands, making scoring more difficult. However, when consistent quantities of DNA template were used, consistent and reproducible results were obtained. Similarly, higher primer concentration gave more bands and unclear bands. The other factor for reproducibility of RAPD bands is the size of amplified products. Amplified products with small size (less than 200 bp) and large size (more than 2000 bp) showed low reproducibility. Good reproducibility was obtained with bands between 250-1500 bp.

Table 2: The matrix of similarity index among individuals of four populations

Locations	Perlis	Perak	Johore	Terengganu
Perlis	0.000			
Perak	0.205	0.000		
Johore	0.625	0.654	0.000	
Terengganu	0.458	0.468	0.614	0.000

Genetic distance and dendrogram: The SI between four populations of snakehead fish ranged 0.1520 - 0.7563. The genetic distances were not far among populations. The average of similarity index is shown in Table 2. The highest value is between Perak and Johore populations. The UPGMA dendrogram among populations is presented in Fig. 2.

DISCUSSION

Using Phenol Chloroform method for extraction of DNA from six species given DNA concentration varied from 35.69 to 71.79 μg μL^{-1}. The determination of optimum concentration in each retractions for PCR analysis were of 0.5 mM dNTP, 4 U μL^{-1} Taq polymerase, 1.5 mM Magnesium chloride and 2.5X buffer. The optimal concentration of Genomic DNA was 25 ng. The optimal annealing temperature for the Operon primers and DNA polymerase in this experiment was found to be 36°C. The number of cycles were kept constant through all analysis, as were denaturation, annealing and extension temperatures; 30 cycles of 94°C for 3 min, 36°C for 2 min and 72°C for 2 min. The amplification reaction ended with 10 min. at 72°C. The current PCR analysis was similar with Dinesh *et al*. (1993) with lower annealing temperature (36°C).

All individuals from each of varieties were grouped in the same cluster. The degree of similarity between individuals was slightly range among four varieties. RAPD fingerprinting is a useful tool for assessment of genetic variability and can be applied to breeding program in aquaculture. Reproductive program is carried out based on similarity coefficient. It can be formulated to increase

genetic variation within brood stocks with high similarity coefficient value by outcrossing other breeders with lower similarity coefficient index (Koh *et al*., 1999).

RAPD fingerprinting is to yield reliable and useful results. There is a risk of misinterpretation in a genetic analysis different RAPD patterns have similar size. This can be minimized by the use of several RAPD primers so that the genetic analyses are based on a large number of pooled RAPD markers (Bidochka *et al*., 1994). In addition, generally many RAPD bands of mixed intensity (i.e., strong or faint, fuzzy or sharp) are generated with each primer, because the target DNAs are undefined, one or more copies of the target DNA may exist per genome of percentage of hybridization of primer to target DNA may vary. The problem of mixed-intensity bands exacerbates the well known sensitivity of PCR to the reaction parameters. The optimization of protocol can be obtained reproducible, interpretable RAPD banding pattern in an organism. According to Dinesh *et al.* (1995). there are two types of problems with PCR products. The first problem is the generation of unreliable RAPD products under identical PCR conditions. The second problem is the use of DNA templates that are not of comparable quality and quantity. The issue of reproducibility of RAPD fingerprints generated by different laboratories was tested by Penner *et al.* (1993). Their study showed that different laboratories, using identical primers, amplified different size ranges in RAPD markers in oat cultivars. They also identified that variation of RAPD profiles is due mainly to the fact that different thermo cyclers can have different temperature cycling profiles. The annealing temperature inside reaction tubes is identical among laboratories due to RAPD fragments are likely to be reproducible (Penner *et al*., 1993). In addition, the use of appropriate gel matrix to obtain good resolution and detection system for visualization of RAPD products is also crucial for gene mapping, genotyping of closely related or highly inbred species and measurement of genetic variation at intraspecific level (Caetano-Anolles *et al*., 1999; Dinesh *et al*., 1995). Generally, any inept DNA pattern generated in the sample because of unsatisfactory amplification or inadvertent working errors should not be included in the data analysis. This present results indicate that good reproducibility can be achieve using high quality primers and for PCR products of 200-1500 bp. Bands greater than 2000 bp should not be scored unless one is sure about the reproducibility of bands with higher molecular weight.

Although one major drawback of the RAPD methodology is the impossibility to determine if an individual is homozygous or heterozygous due to its dominant character, the results showed that the technique stands for a simple and cost-efficient approach to gain information about general genetic variability within and among individuals of *Channa* species. The dendrogam shows that Terengganu and Johore populations are closed comparing with Perlis and Perak (Fig. 2). Following the geography, Terengganu and Johore locate in the eastern Malaysia. In that, Perak and Perlis are in western Malaysia. The west and east are divided by high mountain so genetic relationships also follow the geographical distance.

In addition, the RAPD polymorphism is unclear inheritance patterns. It is not possible to determine if an individual has two fragments occupying the same position on a gel might not be identical. The RAPD technique might not be ideal for genetic studies, but the approach seem useful for identification and phylogenetic studies. A major advantage with RAPD is that the entire procedure is very fast compared with other DNA-based method (*Southern blotting, sequencing, microsatellite*). RAPD analysis can show high levels of polymorphism in species with low electrophoretical (allozyme) variation. Given the technical problems that might occur using RAPD's it is, however, recommended to use this technique only when other easily adapted genetic methods like allozymes, fail to produce polymorphism.

RAPD technology is a useful tool for identifying DNA polymorphism, estimation of genetic diversity and difference of related species in fish. However, it is essential to optimize RAPD amplified condition and ascertain the reproducibility of RAPD markers for individual taxa prior to apply RAPD fingerprinting to any genetic analysis. In common with other molecular techniques, RAPD analysis has been successful to apply in tiger barb (*Puntius tetrazona*) breeding program. Asma (1999) suggested that hybridisation could be carried out between varieties based on green and yellow colour, cultivated and wild stock, and location. Koh *et al.* (1999) mentioned that interbreeding among the varieties of wild and cultivated discus fish (*Symphysodon* spp.) must be careful and could be planned on the basis of classification and the nomenclature and genetic. In current study, the polymorphisms are low between populations which are short geography.

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REFERENCES

Amstrong, J., S. Gibbs, A.J. Peakall and G. Weiller, 2004. RAPDistance Package. http://life.anu.edu.au/molecular/software/rapd.html.

Asma, A.N., 1999. Genetic variation between and within three varieties and domesticated Tiger barb (*Puntius tetrazona*) using RADP markers. M.Sc Thesis, Univ. Putra Malaysia.

Bardakci, F. and D.O.F. Skibinski, 1994. Application of RAPD technique in tilapia fish: Species and subspecies identification. Heredity, 73: 117-123.

Bidochka, M.J., M.A. McDonald, R.J. St Leger and D.W. Robert, 1994. Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA (RAPD). Curr. Genet., 25: 107-113.

Bielawski, J.p. and D.E. Pumo, 1997. Randomly Amplified Polymorphic DNA (RAPD) analysis of Atlantic coast striped bass. Heredity, 78: 32-40.

Caetano-Anollés, G., B.J. Bassam and P.M. Gressoff, 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technol., 9: 553-557.

D'Amato, M.E. and D. Corach, 1996. Genetic diversity of population of the freshwater shrimp *Macrobrachium borellii* (Caridae: Palaemonidae) evaluated by RAPD analysis. Crust. Biol., 16: 650-655.

Dinesh, K.R., T.M. Lim, W.K. Chan and V.P.E. Phang, 1993. RAPD analysis: An efficient method of DNA fingerprinting in fisheries. Zool. Sci., 10: 849-854.

Dinesh, K.R., W.K. Chan, T.M. Lim and V.P.E. Phang, 1995. RAPD markers in fishes: An evaluation of resolution and reproducibility. Asia- Pacific Mole. Biol. Biotechnol., 3: 112-118.

Dinesh, K.R., V.P.E Phang and T.M. Lim, 1996. PCR based DNA fingerprinting using arbitrary primers in few cultivable fish. BIOTROP Spec. Publ., 52: 1118.

Dioh, W., D. Tharreau and M.H. Lebrun, 1997. RAPD based screening of genomic libraries for positional cloning. Nucl. Acid Res., 25: 5139-5131.

Elo, K., S. Ivanoff, J.A. Vuorinen and J. Piironen, 1997. Inheritance of RAPD markers and detection of interspecific hybridization with brown trout and Atlantic salmon. Aquaculture, 152: 55-65.

Fritzch, P. and L.H Rieseberg, 1996. The use of random amplified polymorphic DNA (RAPD) in conservation genetics. Molecular Genetic Approaches in Conservation (Smith T.B. and R.K. Wayne. Oxford Univ. Press, New York.

Hallerman, E.M. and Beckmann, 1988. DNA level polymorphism as a tool in fisheries science. Can. J. Fish. Aqua. Sci., 45: 1075-1087.

Heist, E.J. and J.R. Gold, 1999. Genetic identification of sharks in the U.S. Atlantic large coastal shark fisheries. Fish. Bull., 97: 53-61.

Iturra, P., J.F. Medrano, M. Bagley, N. Lam, N. Vergara and J.C. Marin, 1998. Identification of sex chromosome molecular markers using RAPDs and fluorescent in situ hybridization in rainbow trout. Genetica, 101: 209-213.

Jong-Man, Y., 2001. Genetic similarity and difference between common carp and Israeli carp (*Cyprinus carpio*) based on Random Amplified Polymorphic DNAs analyses. Kor. J. Biol. Sci., 5: 333-339.

Kim, C.L., 1998. Development of PCR-based DNA markers to identify and characterise Malaysian river catfish *Mystus nemurus* (C and V): RAPD and AFLP. M.Sc. Thesis, Fac. Sci. Environ. Stud., Univ. Putra Malaysia.

Koh, T.L., G. Khoo, L.Q. Fan and V.P.E. Phang, 1999. Genetic diversity among wild forms and cultivated varieties of Discus (*Symphysodon* spp.) as revealed by random amplified polymorphic DNA (RAPD) fingerprinting. Aquaculture, 173: 485-497.

Liu, Z.J., P. Li, B.J. Argue and R.A. Dunham, 1999. Random Amplified polynorphic DNA markers: Usefulness for gene mapping and analysis of genetic variation of catfish. Aquaculture, 174: 59-69.

Lynch, M., 1990. The similarity index and DNA fingerprinting. Mol. Biol. Evol., 7: 478-484.

Lynch, M., 1991. Analysis of population genetic structure by DNA fingerprinting. DNA fingerprinting-Approaches and application, Burke, T., G. Dolf, A.J. Jeffeys and R. Wolf, Ed. Basel, Switzerland, pp: 113-126.

Mamuris, Z., A.P. Apostolitis, A.J. Theodorou and C. Triantaphyllidis, 1998. Application of random amplified polymorphic DNA (RAPD) markers to evaluate intraspecific genetic variation in red mullet (*Mullus barbatus*). Mar. Biol., 132: 171-178.

Meruane, J., M. Takagi and M. Taniguchi, 1997. Species identification and polymorphism using RAPD-PCR in penaeid prawn *Penaeus monodon* and *Metapenaeus ensis*. Fish. Sci., 63: 149-150.

Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc. Nat. Acad. Sci., USA, 76: 52699-3263.

Penner, G.A., A. Bush, R. Wise, W. Kim, L. Domier, K. Kasha, A. Laroche, G. Scoles, S.J. Molnar and G. Fedak, 1993. Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Meth. Appl., 2: 341-345.

Saiki, K.K. 1992. The Design and Optimization of the PCR. In: PCR Technology-principles and Applications for DNA Amplification. Erlich H.A., (Ed.) W.H. Freeman and Company, New York, 146: 7-22.

Shima, T.I.S., 1999. PCR-RAPD analysis on DNA of mudskipper, *Periphthalmus schlosseri* (Pallas). M.Sc Thesis, Fac. App. Sci.Technol., Univ Putra Malaysia

Smith, P.J., P.G. Benson and S.M. McVeagh, 1997. Comparison of three genetic methods used for stock discrimination of orange roughy, *Hoplostethus atlanticus*: Allozymes, mitochondrial DNA and Random Amplified Polymorphic DNA. Fish. Bull., 95: 800-811.

Sneath, P.H.A. and R.R Sokal, 1973. Numerical Taxonomy. W.H. Freeman, San Francisco.

Taggart, J.B., R.A. Hynes, P.A. Prodohl and A. Ferguson, 1992. A amplified protocol for routine total DNA isolation from salmonid fishes. Fish Biol., 40: 963-965.

Takagi, M. and N. Taniguchi, 1995. Random amplified polymorphic DNA (RAPD) for identification of three species of *Anguilla*, *A. japonica*, *A. australis* and *A. bicolor*. Fish. Sci., 61: 884-885.