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# MORPHOMETRIC AND GENETIC VARIATIONS OF *ETROPLUS SURATENSIS* (BLOCH) (ACTINOPTERYGII: PERCIFORMES: CICHLIDAE) FROM TWO TROPICAL LACUSTRINE ECOSYSTEMS, KERALA, INDIA

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**Abstract:** Genetic diversity or variation and its measurement have vital importance in interpretation, understanding and management of populations and individuals. We studied the morphometric and genetic variations of *E. suratensis* populations inhabiting two tropical lacustrine ecosystems, Vellayani freshwater lake and Veli brackishwater lake in Kerala state of India. The morphometric characters of Vellayani population showed reasonable variation when compared with Veli population. Out of the 20 primers tested, ten primers were used for RAPD analysis. RAPD banding pattern showed variations between populations and the percentage of polymorphic loci was recorded as 49.02% and 60.78% for fish populations collected from Vellayani and Veli lakes of India, with a gene flow of 2.32. Nei's unbiased measure of genetic identity and genetic distances of two populations were 0.8832 and 0.1242, respectively. The result of RAPD analysis indicates that the genetic variation among Veli population is higher than the Vellayani population. The present data recorded significant phenotypic and genotypic variability of the E. suratensis populations in the two lacustrine ecosystems of India and this can be used to differentiate some of these populations.

***Key words:*** Genetic diversity, RAPD, lake, pearl spot

## INTRODUCTION

One of the three Gondwanan teleost species from India, the Green Chromide *Etroplus suratensis* (Bloch), is distributed in the freshwater and brackishwater bodies of Kerala state of India and in Sri Lanka. It has been introduced to many states along the east and west coasts of India for promoting aquaculture. This fish, declared in 2010 as the state fish of Kerala, is an important food fish and a preferred candidate for brackishwater aquaculture in India. Despite the evolutionary and economic importance of this fish, there are no reports on its morphological heterogeneity and population differentiation in India. Suneetha (2007a) studied the intra-specific phenotypic and genotypic variations of *E. suratensis* in Sri Lanka.

Morphological characters such as morphometrics and meristics have been commonly used to identify stocks of fish (Teugels, 1982; Turan *et al.,* 2004; Suneetha and Damayanthi, 2008) and for establishing the evolutionary linkages between ancient and modern fish fauna (Deesri *et al.,* 2009). In fishery biology morphometric or biometric studies are used to estimate the percentage of fish harvested from length-weight data, to determine the effects of environmental improvement and to regulate fisheries (Analaura *et al.,* 2005).

Understanding of genetic diversity between different population of a species and between closely related species is useful for genetic

management and conservation of endemic populations. Molecular markers can be utilized in the assessment of genetic variation in fish, differentiation of stocks/populations and hence in fisheries management (Hallermann and Beckmann, 1988). Random Amplified Polymorphic DNA (RAPD) markers have been used effectively for studying the genetic variation populations with differential degrees of geographic isolation, especially in fish (Ali *et al.,* 2004). RAPD technique has been applied to the study of phylogenetic relationship in tilapia and other cichlid species (Bardakci and Skibinski, 1994), though this has no far been attempted with *Etroplus suratensis.*

Of late, decline in the natural populations of green chromide has been reported from India, primarily due to anthropogenic interventions such as pollution of water bodies, over exploitation, watershed alterations and creation of barricades, all resulting in ecological degradations of ecosystems (Padmakumar *et al.,* 2002). Hence it is becoming increasingly important to study the existing levels of phenotypic and genetic variation of fish fauna inhabiting various water bodies. The objective of the study is to document the morphometric and genetic variations of two populations of *Etroplus suratensis* inhabiting two closely situated lacustrine ecosystems in Kerala state, India.

## MATERIALS AND METHODS

### Research area and samples

Specimens of *Etroplus suratensis* were collected from Vellayani (8"24'-8"26' N and 76°59-76°59' E) and Veli (8°28'N and 76°57'E) lakes located in Thiruvananthapuram district, Kerala state, India; Vellayani is a freshwater lake, while Veli is a brackishwater lake which maintains connectivity with the sea during certain periods of the year (Fig. 1). Sampling was performed by using gill

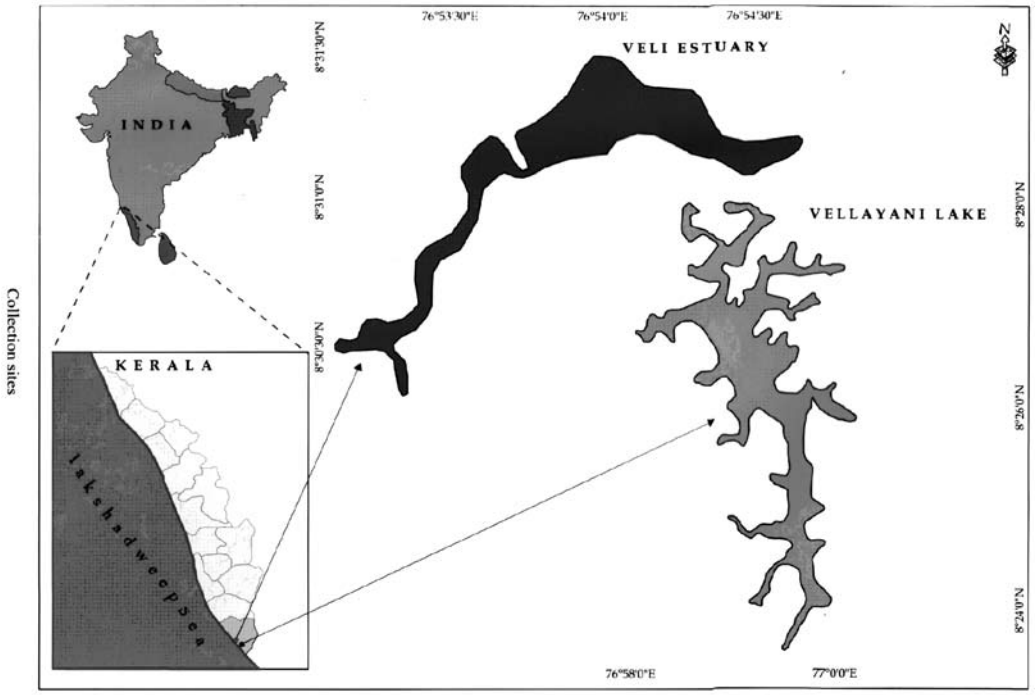


Fig. 1. Map showing the location of Vellayani and Veli lakes in Kerala, India

nets of varying mesh sizes, with the help of local fishermen. A total of 100 fishes were collected from both the locations for morphometric analysis and preserved in 10% formalin for further studies.

## Morphometric analysis

Morphometric and meristic data were gathered following methods described by Strauss and Bond (1990). The meristic characters were counted using magnifying hand lens and the morphometric characters were measured to the nearest millimeter with digital calipers. Data were analysed using statistical package for social sciences SPSS version 11. Mean morphometric and median meristic parameters were compared between two populations of fish using Student's 't' test and non-parametric Mann Whitney U test respectively. To find out the morphometric factors that influence the two populations, Principal Component Analysis (PCA) was used in which factor loadings based on Eigen values were used to determine the morphometric factors.

## *DNA extraction*

Genomic DNA was extracted from the muscle tissues of the fish by the phenol-chloroform procedure (Sambrook *et al.,* 1989). The purity and concentration of genomic DNA were determined by calculating the ratio of the optical density measured at 260-280 nm with a spectrophotometer. Analysis on agarose gels was used to determine the quality of DNA. DNA samples were diluted to approximately 25ngµL-1 with deionised distilled water and used for PCR amplification.

## *RAPD analysis*

Twenty commercially available decamer primers (Operon Technologies) were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD analysis. After initial screening with all 20 primers, 10 primers were selected by detecting the sharp high intensity reproducible bands (Table 1). Amplification was performed in a total volume of 12.5µl containing: 1X Taq polymerase buffer 2mM magnesium chloride 10mM dNTPs primer, 0.5U Taq polymerase and 25ng template DNA. Control reactions were run containing all components except genomic DNA. Amplifications were performed using an thermocycler. The reaction mixture was pre-heated at $95^0c$ for 3 min, followed by 39 cycles ($94^0c$ for 1 min, $40^0c$ for 1 min and $72^0$c for 1.30 min). The reaction was then subjected to a final extension at $72^0c$ for 10 min. The amplification products were size-fractioned in a 1.2% agarose gel containing ethidium bromide in TBE buffer and photographed on a UV transilluminator using a gel documentation system. A low range known DNA marker was run with every gel (Lamda DNA-hind111/Eco R1 digest and/or 1kb DNA ladder, Bangalore Genei, India) to estimate the molecular sizes of the RAPD product.

**Table 1.** Primers and primer sequences used for the detection of polymorphism in *Etroplus suratensis* (Bloch)

| Sl. No. | Primer | Sequence (5'-3') |
|---|---|---|
| 1 | OPA 03 | 5'-AGTCAGCCAC- 3' |
| 2 | OPA 04 | 5' AATCGGGCTG- 3' |
| 3 | OPA 05 | 5' AGGGGTCTTG- 3' |
| 4 | OPA 07 | 5' GAAACGGGTG-3' |
| 5 | OPA 08 | 5'GTGACGTAGG-3' |
| 6 | OPA 10 | 5'GTGATCGCAG- 3' |
| 7 | OPD 03 | 5'GTCGCCGTCA3' |
| 8 | OPD 11 | 5'AGCGCCATTG3' |
| 9 | OPD 18 | 5'GAGAGCCAAC3' |
| 10 | OPD 20 | 5'ACCCGGTCAC3' |

The RAPD bands were scored as present (1) or absent (0) in each pattern. All calculations were carried out using the population genetic analysis software, POPGENE 1.31 (Yeh *et al.,* 1999). The UPGMA dendrogram of population was constructed based on Nei's (1972) and genetic

distances using TFPGA (Tools for Population Genetics Analysis) software (Miller, 1997). Genetic differentiation (Gst) was calculated by using formula: Genetic differentiation (Gst) = 1-Hs/Ht, where Hs is sample gene diversity and Ht is total gene diversity. Gene flow was indirectly estimated among the populations by using the formula: Nm= 0.5 (1-Gst)/Gst (McDermott and McDonald, 1993). Shannon's diversity index was calculated to provide a relative estimate of the degree of genetic variation within each population using POPGENE 1.31 (Yeh, 1999).

## RESULTS AND DISCUSSION

The morphometric and meristic characters of *Etroplus suratensis* populations and their comparison are presented in Tables 2 and 3. Significant variations in total length, standard length, body width, head length, body depth, eye diameter, pre-orbital length, caudal peduncle length, dorsal fin length, dorsal fin base, dorsal fin height, pectoral fin length, pectoral fin base, pelvic fin length, pelvic fin base, anal fin length, anal fin base, caudal fin length, dorsal fin spines, anal fin spines and anal fin rays were observed; morphometric measurements of fishes of Veli brackishwater lake were found higher than the fishes of Vellayani freshwater lake, Kerala, India. PCA revealed that several morphometric parameters play important role in differentiating *Etroplus suratensis* of Vellayani and Veli lakes. PC1 (85.513%) coefficients were all positive, indicating no shape variations between both the populations (Table 4). Total length, standard length, anal fin base, dorsal fin base and body width were the characteristics most highly correlated with PC1. Snout length, caudal peduncle depth and pelvic fin length were the morphometric characters highly correlated with PC2, PC3 and PC4 respectively.

Table 2. Comparison of morphometric parameters between *Etroplus suratensis* (Bloch) populations from Vellayani and Veli lakes of India

| | Component Factor Loadings | | | |
|---|---|---|---|---|
| Parameters | 1 | 2 | 3 | 4 |
| Total Length (mm) | 0.992 | 0.008 | -0.047 | -0.033 |
| Standard Length (mm) | 0.987 | 0.014 | -0.050 | -0.047 |
| Weight (mg) | 0.910 | 0.028 | -0.138 | 0.001 |
| Body Width (mm) | 0.981 | 0.045 | -0.018 | 0.004 |
| Head Length | 0.956 | 0.056 | -0.054 | -0.102 |
| Body Depth | 0.944 | 0.044 | -0.049 | -0.005 |
| Eye Diameter | 0.897 | 0.052 | 0.150 | -0.175 |
| Pre orbital Length | 0.976 | 0.052 | -0.066 | -0.009 |
| Dorsal Fin Length | 0.899 | 0.024 | -0.157 | 0.179 |
| Dorsal Fin Base | 0.982 | 0.039 | -0.060 | -0.042 |
| Dorsal Fin Height | 0.945 | 0.089 | -0.104 | 0.041 |
| Pectoral Fin Length | 0.892 | -0.150 | -0.082 | 0.056 |

| | | | | |
|---|---|---|---|---|
| Pectoral Fin Base | 0.817 | -0.500 | -0.200 | -0.019 |
| Pelvic Fin Length | 0.753 | -0.229 | 0.423 | 0.432 |
| Pelvic Fin Base | 0.634 | 0.742 | 0.126 | 0.073 |
| Anal Fin Length | 0.968 | -0.012 | -0.073 | 0.069 |
| Anal Fin Base | 0.986 | 0.006 | -0.014 | 0.004 |
| Caudal Fin Length | 0.977 | 0.021 | -0.034 | 0.009 |
| Caudal Peduncle Length | 0.885 | -0.068 | 0.199 | -0.081 |
| Caudal Peduncle Depth | 0.804 | -0.171 | 0.430 | -0.286 |
| Eigen Value | 16.703 | 0.933 | 0.568 | 0.369 |
| Percentage of Variance | 83.513 | 4.664 | 2.841 | 1.843 |
| Cumulative Percentage Variance | 83.513 | 88.177 | 91.018 | 92.861 |

According to Jolicoeur and Mosimann (1960), any component having all coefficients (component loadings) of the same sign was indicative of the size variation, whereas any component having both positive and negative coefficients was indicative of shape variation. The coefficients obtained for PC1 were of the same sign and therefore it can be assumed that the populations of *Etroplus suratensis* could be distinguished by size rather than shape variation. Morphometrics and meristics have been commonly used to distinguish the species

**Table 3.** Comparison of meristic parameters between *Etroplus suratensis* (Bloch) populations from Vellayani and Veli lakes, India

| Parameters | Vellayani | | | Veli | | | Total Population | | | Mann Whitney U Value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Median | ± SD | Mean | Median | ± SD | Mean | Median | ± SD | |
| Dorsal Fin Spines | 18.02 | 18.00 | 0.45 | 17.69 | 18.00 | 1.17 | 17.85 | 18.00 | 0.90 | 4352.00* |
| Dorsal Fin Rays | 13.54 | 14.00 | 0.58 | 13.39 | 13.00 | 1.10 | 13.47 | 13.00 | 0.88 | 4571.00 |
| Pectoral Fin Rays | 13.63 | 14.00 | 1.46 | 13.82 | 14.00 | 1.25 | 13.73 | 14.00 | 1.36 | 4853.50 |
| Pelvic Fin Spines | 1.00 | 1.00 | 0.00 | 1.00 | 1.00 | 0.00 | 1.00 | 1.00 | 0.00 | 5100.00 |
| Pelvic Fin Rays | 5.00 | 5.00 | 0.00 | 5.00 | 5.00 | 0.00 | 5.00 | 5.00 | 0.00 | 5100.00 |
| Anal Fin Spines | 12.03 | 12.00 | 0.41 | 11.60 | 12.00 | 0.72 | 11.81 | 12.00 | 0.63 | 341.00** |
| Anal Fin Rays | 10.87 | 11.00 | 0.56 | 11.10 | 11.00 | 0.86 | 10.99 | 11.00 | 0.74 | 4348.50* |
| Caudal Fin Rays | 16.00 | 16.00 | 0.00 | 16.00 | 16.00 | 0.00 | 16.00 | 16.00 | 0.00 | 5100.00 |

**P<0.01; *P<0.05

taxonomically, to identify stocks of fish, and to separate different morphotypes (Doherty and McCarthy 2004; Jayasankar *et al.,* 2004). In general, the morphometric and meristic features of *Etroplus suratensis* populations studied from both the lakes agree with those in taxonomic compilations (Jayaram, 1999). Among the vertebrates, phenotypic variability is considered to be greatest in fish, which have relatively higher within population coefficients of variation of

phenotypes (Carvalho, 1993).

The results established heterogeneity in morphology among populations of *Etroplus suratensis* inhabiting different lacustrine ecosystems. The existence of significant population level differences has previously been noted in estuarine fishes using both morphological and genetic criteria (Roby *et al.,* 1991, Uiblein, 1995, Suneetha, 2007b). The results of this paper are in corroboration with the observation by Suneetha (2007a) in Sri Lanka that *Etroplus suratensis* populations inhabiting various ecosystem maintain morphological heterogeneity and the morphological variation can be used to differentiate some of these populations. Morphometric characters can show high plasticity in response to differences in environmental conditions such as food, abundance, salinity and temperature. Fishes inhabiting both open hydrological systems like estuaries and closed inland lakes have been adapted to maintain their stocks within the system, resulting in some degree of isolation and an identifiable phenotypic differentiation (Suneetha, 2007b). Isolation for longer periods of time and subsequent adaptations could be responsible for the observed variations in morphometry of the two populations of *E. suratensis.*

The RAPD profile of bands obtained in the two populations with 3 primers (OPA 04, OPA 07 and OPD 03) is shown in the Fig. 2. The percentage of polymorphic loci was 49.02 and 60.78 for fish populations of Vellayani and for Veli lakes respectively. The Shannon index ranged from 0.2962 (Vellayani) to 0.3121 (Veli). Polymorphic loci indicate that the genetic variation among Veli populations was higher than that in the Vellayani populations. Reduction of genetic variability may cause greater sensitivity to environmental changes and eventually lead to

**Table 4**. PCA of transformed morphometric variables for Etroplus suratensis (Bloch) populations of Vellayani and Veli lakes, India

| Parameters | Vellayani lake | | | Veli lake | | | Total population | | | Student's t value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Median | ± SD | Mean | Median | ± SD | Mean | Median | ± SD | (comparing lakes) |
| Total Length (mm) | 135.55 | 138.55 | 26.32 | 78.54 | 64.65 | 44.01 | 106.77 | 95.45 | 46.16 | 11.147** |
| Standard Length (mm) | 102.93 | 105.00 | 20.03 | 61.89 | 50.89 | 35.03 | 82.20 | 72.75 | 35.17 | 10.195** |
| Body width (mm) | 61.54 | 63.98 | 11.70 | 35.36 | 29.34 | 20.39 | 48.32 | 42.13 | 21.17 | 11.161** |
| Head length(mm) | 34.44 | 35.58 | 6.57 | 20.54 | 16.91 | 10.23 | 27.42 | 23.50 | 11.07 | 11.466** |
| Body Depth (mm) | 23.71 | 24.50 | 6.38 | 13.99 | 10.47 | 11.18 | 18.80 | 15.98 | 10.32 | 7.572** |
| Eye Diameter (mm) | 8.71 | 8.93 | 1.25 | 5.10 | 4.58 | 1.91 | 6.88 | 6.81 | 2.42 | 15.856** |
| Pre-orbital Length (mm) | 14.99 | 15.21 | 3.47 | 8.93 | 6.99 | 5.40 | 11.93 | 12.24 | 5.46 | 9.474** |
| Caudal peduncle Length (mm) | 6.26 | 6.42 | 1.54 | 3.63 | 2.84 | 2.66 | 4.93 | 4.52 | 2.54 | 8.575** |
| Caudal Peduncle Depth (mm) | 4.86 | 4.97 | 1.29 | 4.72 | 2.62 | 13.36 | 4.79 | 3.63 | 9.51 | 0.104 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dorsal Fin Length | 94.96 | 106.25 | 26.25 | 56.94 | 46.00 | 38.33 | 80.71 | 68.00 | 40.71 | 10.370** |
| Dorsal Fin Base | 68.11 | 71.50 | 14.28 | 39.79 | 32.65 | 23.23 | 53.81 | 48.72 | 23.94 | 10.413** |
| Dorsal Fin Height | 25.20 | 26.16 | 6.47 | 11.29 | 8.28 | 9.74 | 18.18 | 14.03 | 10.81 | 11.939** |
| Pectoral Fin Length | 31.22 | 32.50 | 7.16 | 17.50 | 14.00 | 11.78 | 24.29 | 21.50 | 11.93 | 9.983** |
| Pectoral Fin Base | 8.74 | 9.16 | 2.26 | 5.16 | 4.48 | 2.66 | 6.93 | 5.65 | 3.05 | 10.307** |
| Pelvic Fin Length | 24.51 | 24.60 | 7.34 | 14.22 | 12.50 | 7.93 | 19.31 | 17.75 | 9.21 | 9.565** |
| Pelvic Fin Base | 5.29 | 5.20 | 1.72 | 3.56 | 2.75 | 3.16 | 4.42 | 3.96 | 2.69 | 4.840** |
| Anal Fin Length | 86.65 | 88.00 | 20.63 | 46.23 | 36.50 | 35.11 | 66.24 | 55.50 | 35.21 | 9.950** |
| Anal Fin Base | 48.25 | 49.76 | 9.80 | 28.59 | 23.91 | 16.78 | 38.32 | 33.63 | 16.91 | 10.142* * |
| Caudal Fin Length | 32.34 | 33.11 | 6.52 | 17.28 | 13.79 | 10.29 | 24.74 | 21.90 | 11.45 | 10.370** |

** P< 0.01

**Table 5.** Nei's unbiased measures of genetic identity and genetic distance between *Etroplus suratensis* (Bloch) populations of Vellayani and Veli lakes, India

| Pop ID | Vellayani | Veli |
|---|---|---|
| Vellayani | **** | 0.8832 |
| Veli | 0.1242 | **** |

extinction of a species (Guttman and Berg 1998, Oliveira *et al.* 2002, Lopera- Barrero *et al.,* 2006). Moreover, it may affect growth and reproduction (Porta *et al.,* 2006). Therefore, the maintenance of genetic variability is very important for the conservation of a species (Barroso *et al.* 2005). It is necessary for individuals to have the ability to survive environmental variations and develop fully (Ryman *et al.,* 1995).

Nei's gene diversity was found higher in the fish population of Veli lake (0.2200) than that of Vellayani lake (0.2058). Das *et al.* (2005) observed the varied range of 42.6%, 31.7%, 30%, 19.2%, 16.8% and 14.3% polymorphic loci in different carp species of the genus Labeo. Li and Chu-Wu (2006) calculated very high (86.00-92.11%) polymorphic loci ratio in five species of snappers using the RAPD technique. We recorded less polymorphism of alleles in comparison to the previous reports, which however, documented inter-specific variations.

The total gene diversity (Ht) in the population was 0.0347 and the genetic diversity within population (Hs) was 0.0327. The genetic differentiations (Gst) of all populations were 0.1773 and the gene flows between populations were 2.32. Lower differentiation rate between populations were observed which is very common for RAPD data as the regions of RAPD are expected to be less responsive to selection and to have higher tolerance to mutation as RAPD bands arise from both coding and non-coding regions (Williams *et al.,* 1990). Gene flow among subpopulations is a characteristic attribute of population genetic studies. With high levels of migration and gene flow between populations, the similarity of populations increases (Nigel, 1997).

The high rate of gene flow indicate the migration of *Etroplus suratensis* due to mutation, genetic drift or other activities such as fishing gear, destruction of habitat, alteration of prey availability or pollution stress. Using RAPD data the genetic distance between populations of *E. suratensis* was found to be 0.1242 (Table 5)

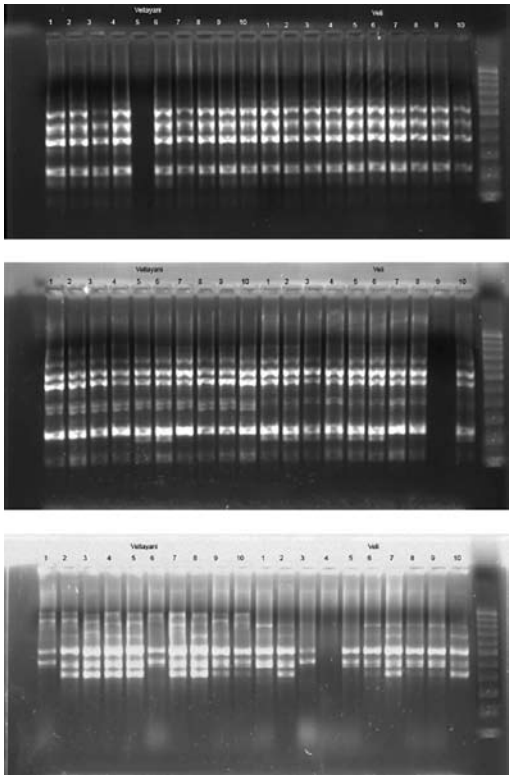

Fig. 2. RAPD banding patterns in *Etroplus suratensis* (Bloch) populations in Vellayani and Veli lakes of India using random primers OPA 04, OPA 07 and OPD 03

and the dendrogram showed one cluster, using RAPD markers.The genetic identity between the populations was 0.8832 (Table 5).

The development of RAPD technique has provided a useful tool for research into genetic variability (Hadrys *et al.,* 1992), population genetics (Lu and Rank, 1996), species and subspecies identification (Bardakci and Skibinski 1994), phylogenetics, linkage group identification, chromosome and genome mapping, analysis of interspecific gene flow and hybrid speciation, analysis of mixed genome samples (Hadrys *et al.,* 1992), breeding analysis and as a potential source for single-locus genetic fingerprints (Brown and Epifanio, 2003). RAPD analysis has been used to evaluate genetic diversity for species, subspecies and population/stock identification in guppy (Foo *et al.,* 1995), tilapia (Bardakci and Skibinski, 1994), brown trout and Atlantic salmon (Elo *et al.,* 1997), largemouth bass (Williams *et al.* 1998), ictalurid catfishes (Liu and Dunham, 1998), common carp (Bartfai e*t al.,* 2003), Indian major carps (Barman *et al.,* 2003) and in *Mystus vittatus* (Garg *et al.,* 2009). Naish *et al.* (1995) found the technique useful in detecting diversity within and between strains of *Oreochromis niloticus.*

Characterization of genetic diversity is a necessary requirement for the improvement, use and conservation of genetic resources. The general goals of population genetic studies are to characterize the extent of genetic variation within species and account for this variation (Weir, 1996). The amount of genetic variation within and between populations can be determined by the frequency of genes and the forces that affect their frequencies, such as migration, mutation, selection and genetic drift (Gall, 1987). Maintaining genetic diversity has become a major issue in conservation biology as it is generally thought to be important for the overall species viability and the potential for evolutionary responses to environmental change (Meffe and Carroll, 1997). Loss of genetic diversity could lead to a decline in ability of a species to cope with changing environment and demographic fluctuations both in the short and long term (Milligan *et al.,* 1994).

In conclusion, the comparative results from both morphological and genetic analysis revealed a reasonable degree of variation in populations of *E. suratensis* in Veli and Vellayani lakes in Kerala, India. In future, additional methods such as microsatellite and sequence analysis can be used to maximize the efficiency of the study. Extensive phenotypic and genotypic studies of this valuable food fish, using individuals from a wide array of habitats, would facilitate their conservation and management programmes in feral water bodies and help identification of better stocks for artificial propagation.

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## REFERENCES

Ali, B.A., Huang, T.H., Qin Da, N. and Wang, X.M. 2004. A review of random amplified polymorphic DNA (RAPD) markers in fish research. *Rev. Fish Biol. Fish.,* 14: 443-453.

Analaura, I.A., Esther, C.S., Manuel, G.C. and Elaine, E.B. 2005. Comparative morphometrics of two populations of *Mugil curema* (Pisces: Mugilidae) on the Atlantic and Mexican Pacific coasts.

Bardakci, F. and Skibinski, D.O.F. 1994. Application of the RAPD technique in Tilapia fish: species and subspecies identification. *J. Hered.,* 73: 117-123.

Barman, H., Barat, A., Bharat, M., Banerjee, Y., Meher, P., Reddy, P. and Jana, R. 2003. Genetic variation between four species of Indian major carps as revealed by random amplified polymorphic DNA assay. *Aquaculture,* 217: 115-123.

Barroso, R.M., Hilsdorf, A.W.S., Moreira, H.L.M., Cabello, P.H. and Traub-Cseko, Y. 2005. Genetic diversity of wild and cultured populations of *Brycon opalinus* (Cuvier, 1819) (Characiforme, Characidae, Bryconiae) using microsatellites. *Aquaculture,* 247: 51-65.

Bartfai, R., Egedi, S., Yue, G.H., Kovacs, B., Urbanyi, B., Tamas, G., Horvath, L. and Orban, L. 2003. Genetic analysis of two common carp broodstocks by RAPD and microsatellite markers. *Aquaculture,* 219: 157-167.

Brown, B. and Epifanio, J. 2003. Nuclear DNA. In: Hallerman, E.M. (ed.), Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland, pp. 101-123.

Carvalho, G.R. 1993. Evolutionary aspects of fish distribution: genetic variability and adaptation. *J. Fish. Biol.,* 43: 53-73.

Deesri, U., Cavin, L., Claude, J., Suteethorn, V. and Yuangdetkla, P. 2009. Morphometric and taxonomic study of a ray-finned fish assemblage (Lepidotes buddh abutrensis, Semionotidae) from the late Jurassic-earliest Cretaceous of NE Thailand. Geological Society of London Special Publication, 315: 115-124.

Doherty, D. and McCarthy, T.K. 2004. Morphometric and merisitic characteristics analysis of two western Irish populations of Arctic Char, *Salvelinus alpilus* (L.). *P. Roy. Irish. Acad.,* 104B: 75-85.

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

Foo, C.L., Dinesh, K.R., Lim, T.M., Chan, W.K. and Phang, V. P.E 1995. Inheritance of RAPD markers in the guppy fish, *Poecilia reticulata. Zool. Sci.,* 12: 535.

Gall, G.A.E. 1987. Inbreeding. In: Ryman and Utter, F.M. (eds.). Population genetics and fishery management. University of Washington, Washington, pp. 47-88.

Garg, R.K., Silawat, N., Pramod, S., Neetu, V. and Mehrotra, N.N. 2009. RAPD analysis for genetic diversity of two populations of Mystus vittatus (Bloch) of Madhya Pradesh, India. *Afr. J. Biotechnol.,* 8(17): 4032-4038.

Guttman, S.I. and Berg, D. 1998. Changes in the genetic diversity of aquatic organisms in the great lakes: causes and consequences. *Setac News.,* 23-24.

Hallermann, E.M. and Beckmann, J.S. 1988. DNA level polymorphisms as a tool in fisheries science. *Can. J. Fish. Aquat. Sci.,* 45: 1075-1087.

Hardys, H., Balick, M. and Schierwater, B. 1992. Amplification of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol., 1:* 55-63.

Jayaram, K.C. 1999. *The Freshwater Fishes of the Indian Region.* Narendra Publishing Company, Delhi.

Jayasankar, P., Thomas, P.C., Paulton, M.P. and Mathew, J. 2004. Morphometric and genetic analysis of Indian Mackerel *(Rastrelliger kanangurta)* from Peninsular India. *Asian Fish.Soci.,* 17: 201-215.

Jolicoeur, P. and Mosimann, J.E. 1960. Size and shape variation in the painted turtle: a principle component analysis. *Growth.,* 24: 691-699.

Li, L. and Chu-Wu, L. 2006. Genetic diversity and molecular markers of 5 snapper species. *J. Agricul. Biotechnol.,* 14: 349-355.

Liu, Z.J., Li, P., Argue, B. and Dunham, R. 1998. Inheritance of RAPD markers in channel catfish *(Ictalurus punctatus),* blue catfish (I. furcatus) and their F1, F2 and backcross hybrids. *Anim. Genet.,* 29: 58-62.

Lopera-Barrero, N.M., Riveiro, R.P., Sirol, R.N., Povh, J.A., Gomes, P., Vargas, L. and Streit, J.R. 2006. Genetic diversity in piracanjuba populations

(Brycon orbignyanus) with the RAPD (Random Amplified Polymorphic DNA) markers. *J. Anim. Sci.,* 84: 170-178.

Lu, R. and Rank, G.H. 1996. Use of RAPD analysis to estimate population genetic parameters in the Alfalfa leaf cutting bee, *Megachite rotundate. Genome.,* 39: 655-663.

McDermott, J.M. and McDonald, B.A. 1993. Gene flow in plant pathosystems. *Annu. Rev. Phytopathol.,* 31: 353-373.

Meffe, G.K. and Carroll, C.R. 1997. *Principles of Conservation Biology.* Sinauer Associates, Sunderland.

Miller, M.P. 1997. Tools for population genetic analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by the author.

Milligan, VG., Leebens-Mack, J. and Strand, A.E. 1994. Conservation genetics: beyond the maintenance of marker diversity. *Mol. Ecol.,* 3: 423-435.

Naish, K.A., Warren, K., Bardakci. F., Skibinski. D.O.F., Carvalho, G.R. and Mair, G.C. 1995. Multilocus DNA finger printing and RAPD reveal similar genetic relationships between strains of Oreochromis niloticus (Pisces: Cichlidae). *Mol. Ecol.,* 4: 271-274.

Nei, M. 1972. Genetic distance between populations. *Am. Nat.,* 106: 283-292.

Neigel, J.E 1997. A comparison of alternative strategies for estimating gene flow from genetic markers. *Annu. Rev. Ecol. Syst.*, 28: 105-128.

Oliveira, A.V., Prioli, J.A., Prioli, S.M.A.P., Pavanelli, C.S., Julio, H.F. and Panarari, R.S 2002. Diversity and genetic distance in populations of *Steindachnerina* in the Upper Parana river floodplain. *Genetica,* 115: 259-257.

Padmakumar, K.G., Krishnan, K., Manu, P.S., Shiny, C.K. and Radhika, R. 2002. Thanneermukkom barrage and fishery decline in Vembanad wetlands, Kerala. In: Jayakumar, M. (ed.), *Wetland conservation and Management in Kerala;* State Committee on Science, Technology and Environment. Thiruvananthapuram, Kerala, India, pp. 27-36.

Porta, J., Porta, J.M., Martinez-Rodriguez and Alvare, M.C. 2006. Development of a microsatellite multiplex PCR for Senegalese sole *(Solea senegalensis)* and its application to broodstock management. *Aquaculture.,* 256: 159-166.

Roby, D., Lambert, J.D. and Sevigny, J.M. 1991. Morphometric and electrophoretic approaches to discrimination of capelin *(Mallotus villosus)* populations in the estuary and Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.,* 48: 2040-2050.

Ryman, N., Utter, F. and Laikre, L. 1995. Protection of intraspecific biodiversity of exploited fishes. *Rev. Fish Biol. Fish.,* 5: 417-446.

Sambrook, J., Fritsch, E.F. and Maniatis T. 1989. *Molecular Cloning, A laboratory Manual.* 2nd ed. Gold Spring Harbor Laboratory Press, New York.

Strauss, R.E. and Bond, C.E. 1990. Taxonomic methods: morphology. In: Moyle, P. and Schreck, C. (eds.), *Methods for Fish Biology,* American Fisheries Society, Special Publication, pp. 109-140.

Suneetha, G.K.B. 2007a. Morphological heterogeneity and population differentiation in the green chromide *Etroplus suratensis* (Pisces: Cichlidae) in Sri Lanka. *Ruhuna Journal of Science.,* 2: 70-81.

Suneetha, G.K.B. 2007b. Morphological heterogeneity in some estuarine populations of the cat fish Arius jella (Ariidae) in Sri Lanka. Ceylon. *J. Sci. Biol. Sci.,* 36(2): 100-107.

Suneetha, G.K.B. and Damayanthi, H.G.B.N. 2008. Morphometric and isozyme confirmation for species level divergence between *Puntius dorsalis* (Pisces: Cyprinidae) and its presumed red-fin variety in Sri Lanka. *Ruhuna Journal of Science,* 3: 25-34.

Teugels, G.G. 1982. Preliminary results of a morphological study of five African species of the subgenus *Clarias* (Pisces: Clariidae). *J. Nat. Hist.,* 16(3): 439-464.

Turan, C., Erguden, D., Turan, F. and Gurlek, M. 2004. Genetic and morphological structure of *Liza abu* (Heckel, 1843) populations from the rivers Orontes, Euphrates and Tigris. *Turk. J. Vet. Anim. Sci.,* 28: 729-734.

Uiblein, F. 1995. Morphological variability between populations of *Neobythites stefanov*i (Pisces: Ophidiidae) from deep red sea and Gulf of Aden. *Mar. Ecol. Prog. Ser.,* 124: 23-29.

Ward, J.A. and Wyman, R.L. 1977. Ethology and Ecology of the cichlid fishes of the genus *Etroplus* in Sri Lanka: Preliminary findings. *Env. Biol. Fish.,* 2(2): 137-145.

Weir, V.S. 1996. Intraspecific differentiation. In: Hillis, D.M., Moritz, C. and Mable, B.K. (eds.), *Molecular Systematics,* Sinauer Associates, Massachusetts, pp. 385-406.

Williams, D.J., Kazianis, S. and Walter, R.B. 1998. Use of random amplified polymorphic DNA (RAPD) for identification of largemouth bass subspecies and their intergrades. *Trans. Am. Fish. Soc.,* 127: 825-832.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Reafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. N*ucleic Acids Research* 18: 6531-6535.

Yeh, F.C., Yang, R.C. and Boyle, T. 1999. POPGENE version 1.31, The Microsoft Window based freeware for Population Genetic Analysis, Edmanton, Alberta: University of Alberta.