Molecular Genetics of *Tenualosa ilisha* and a few ornamental fishes

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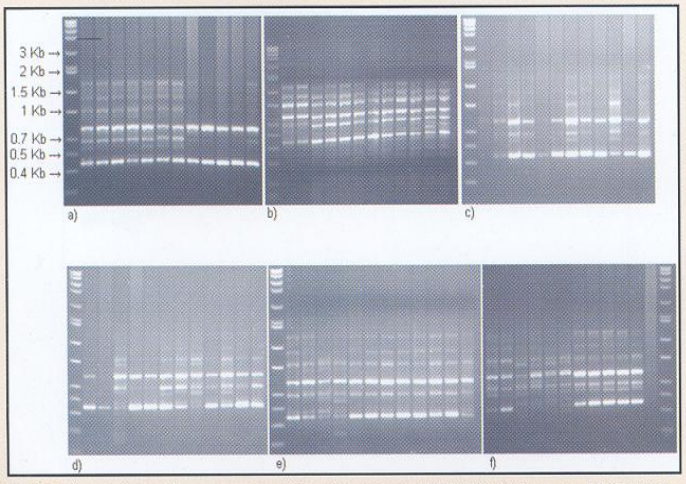
Tenualosa ilisha is an anadromous fish occurring in Indo-West Pacific region in the foreshore areas, estuaries, brackish water lakes, and freshwater rivers. It ascends rivers for breeding during monsoon season and returns to sea after completion of spawning to marine habitats. Hilsa is an important tropical fish belonging to family Clupeidae and sustains a highly commercial fishery in Ganga and Hooghly estuaries in India. All hilsa stocks appear to breed in upper reaches where eggs, larvae and juveniles are found during the spawning seasons. During southwest monsoon, which forms the main breeding season, all early stages can be found in the spawning grounds. The normal habitat of hilsa is lower regions of the estuaries and foreshore areas. During breeding season they ascend rivers and after spawning return to the original habitat where they remain until next breeding season. The upstream migration during main breeding season appears to depend largely on the commencement of southwest monsoon and consequent flooding of rivers. The variations in the intensity of monsoon during breeding season appear to cause considerable fluctuations in abundance of the fish and catches in different places. In India, hilsa distribution has been recorded from the Narmada and Tapti rivers and from Vembanad backwaters of western India. In eastern region hilsa is distributed in Cauvery, Krishna, Godavari, Mahanadi, Hooghly, and Ganga rivers. In 1873, Day described two classes of hilsa from the rivers, a) one-year-old hilsa appearing not to breed and b) those breeding at the start and during monsoon. Mojumdar, 1939 recognized three ecotypes of hilsa; from saline water of sea, muddy freshwater and clear freshwater. Pillay *et. al.* 1963 differentiated three stocks of hilsa using biometrical studies. Based on morphological characteristics, Ghosh *et. al*. 1968 and Quddus *et. al.* 1984 differentiated hilsa into slender and broad morphotypes. Pillay *et. al.* 1963 concluded that hilsa populations of Hooghly, Padma and Ganga show little or no movement between rivers, with little intermingling of populations. Thus, to gain insight into the structure of hilsa populations spawning in Indian rivers, RAPD technique was used to delineate its populations.

Hilsa from the rivers Ganga, Yamuna, Hooghly from the east flowing rivers and from west flowing Narmada and Tapti were sampled. Six oligodecamer primers, OPA-10, OPA-11, OPA-19, OPC-01, OPD-11, and OPD-19 were employed for generating RAPD fragment patterns for samples from each location. This DNA fragment data was analyzed using population genetic programmes TFPGA and PopGene 32. The highest genetic distance was found between Yamuna and Narmada at Allahabad and Bhadbhud respectively (0.394). Lowest genetic distance values were between Allahabad and Lalgola (0.213). UPGMA dendrogram based on Nei (1972) genetic distance indicated the segregation of ***Tenualosa ilisha*** populations collected from six different sites in two clusters; Allahabad (Yamuna), Beniagram (Ganga), Lalgola (Ganga) belonged to Ganga-Padma cluster and Feeder Canal, Nawabganj (Hooghly) and Bhadbhud (Narmada) belonged to Hooghly-Matlah cluster. The average genetic distance for two groups was 0.319. The within group average genetic distance for Ganga cluster was 0.240 and for Hooghly cluster was 0.277. Differences were observed within the two clusters for each population. The overall average genetic distance in all six locations was 0.295. The study shows existence of genetic variation within and between hilsa populations of rivers of India indicating presence of separate stocks or races of hilsa that may be due to the differences in the river ecology, spawning grounds, nursery grounds of the juveniles,



RAPD fragment patterns generated using primer OPA-11 a) Yamuna-Allahabad, b) Ganga-Beniagram, c) Ganga-Lalgola, d) Feeder Canal-Farakka, e) Hooghly-Nawabganj, f) Narmada-Bhadbhud. Lane 1 is wide range DNA marker

seasonal migration and natal behavior of anadromous clupeids.

Unweighted pair group method of arithmetic mean (UPGMA) dendrogram on differentiation between *Tenualosa ilisha*

The mitochondrial DNA 16S ribosomal RNA gene region was partially amplified using cross-species primers which yielded a PCR amplicon of 1.3 Kb size. The DNA sequencing was carried out and sequence submitted to the NCBI gene bank with accession no. DQ400344.

CTGAATCTAGCCGTAACTTCAGCTTGGAGTTCTCTAGTTTGAGCCC
CCGAAACCGGACGAGCTACTCCGGGACAGCCTAACGTAGGGCCAA
CCCGTCTCTGTGGCAAAAGAGTGGGAAGATCCCCGAGTAGAGGTG
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AGAAGTTCAGCTCCGTCAAGCGCCCTTCCCCCGATCCCGATCAAAC
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CATGGGAGAGACCATGCTAGAACGAGTAATAAGAAGAAAGAACTTC
TCCCCGCACATGTGTAAGTCGAATCGGACCACCCATCGACGATTAA
CGAACCCAACAACAGAGGCCCATGCACCATCGCCACGCAGGCCAA
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CCTAACCGTGCAAAGGTAGCGCAATCAATTGTCTTTTAAATGGAGA
CCTGTATGAATGGCATAACGAGGGTCTAACTGTCTCTTTTTTCCAGT
CAATGAAACTGATCTGGCCGTGGGGGGGCGGGCATGAGTGCACAA
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GCAGCCCCACTAATAGACCTCCAAATAAGATGGGCCCTGGTATAAA
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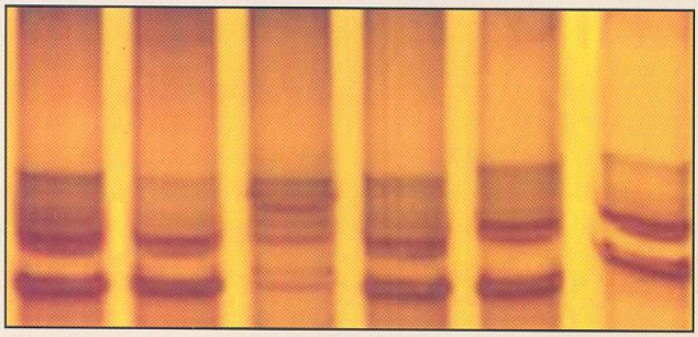
TCGACGAGAGGGTTTACGACCTCGATGTTGATCGGGACATCCTAAT
GGTGCAGCCGCTATTAAGGGTTCGTTTGTCACGATTAGAGTCCTAC
GTGATCTGAGTTCAAACCGG

1.3 Kb DNA sequence of the mitochondrial 16S rRNA gene of *Tenualosa ilisha*

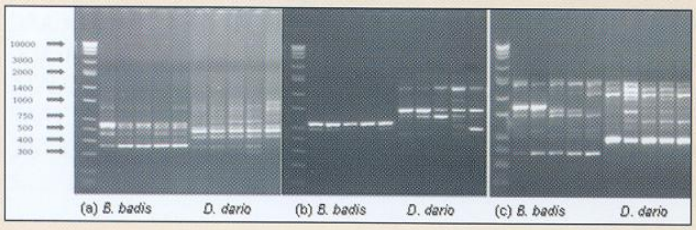
A 360 bp region of mitochondrial DNA cytochrome b gene was amplified using cross species primers. Single Stand Conformational Polymorphism (SSCP) was used to distinguish between polymorphic DNA haplotypes. A total of 21 SSCP haplotypes were identified and were DNA sequenced. DNA sequence data indicates haplotypic variation in hilsa populations.



SSCP haplotypes of the mitochondrial Cytochrome b gene of *Tenualosa ilisha*

Molecular Taxonomy of *Badis badis* and *Derio derio*

Molecular identification and taxonomic resolution using molecular markers was employed for ***Badis badis*** and ***Dario dario*** species which have undergone a series of revisions

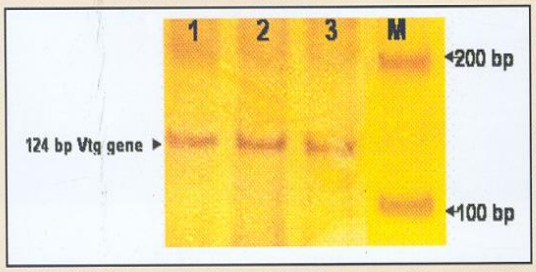


RAPD patterns of Badis badis and Dario dario using degenerate primer

in their taxonomic status at genus and species level. In 1822, Hamilton identified these fishes under the genus ***Labrus*** and named them as ***Labrus badis*** and ***Labrus dario*** respectively (Hamilton, 1822). Kullander and Britz (2002) recently revised the family Badidae and placed ***Badis badis bengalensis*** in a new genus ***Dario***. RAPD was used to generate the species-specific diagnostic fragment patterns for molecular identification of the ornamental aquarium fish species ***Badis badis*** and ***Dario dario***. Seven arbitrary primers produced a total of 116 bands of which 98.23% were polymorphic. The size range of the amplified products was in the range of 340 bp to 2170 bp. The dendrogram displayed two distinct clusters for the two species indicating their separate evolutionary status.

Pollution Biomarker Gene Expression

Biomarker gene expression studies was conducted for, Vitellogenin gene in ***Labeo rohita*** exposed to endocrine disruptor 17 ß Estradiol, using cross species gene primers from ***Barbus barbus*** and ***Cyprinus carpio***. These gene expression markers were analysed using Reverse Transcriptase-PCR method. A 124 bp region of vitellogenin cDNA was PCR amplified. This maker serves as rapid and early indicator of stress in fishes exposed to endocrine disruptors.



Vitellogenin gene expression at higher to lower concentrations of Copper Sulphate