ABSTRACT

Badis badis or Chameleon Fish (Hamilton-Buchanan, 1822) (Actinopterygii, Perciformes, Badidae) and *Amblyceps mangois* or Indian Torrent Catfish (Hamilton-Buchanan, 1822) (Actinopterygii, Siluriformes, Amblycipidae) are two a tropical, freshwater species that has ornamental importance and thus possesses commercial value. The species has been categorized as vulnerably susceptible in the list of threatened freshwater fishes of India by NBFGR, Lucknow, India- country's apex body for the purpose. However, studies with regard to genetic diversity of fish species especially in the eastern sub-Himalayan region of West Bengal, India have been found to be very inadequate. On the standpoint of validating its threatened status, assessment of its genetic diversity from this particular region with the efforts to conserve its wild population seem to be of great significance.

Genetic variability of an organism is of paramount importance from the standpoint of survival and reproductive fitness as well as for sustenance of the whole population; therefore, the erosion of genetic diversity of a population curtails its capability for adaptation and increases the risk of its extinction. The vulnerable organisms having small/minute population size may experience a perpetual reduction in the genetic variation. However, maintaining the genetic variation in a threatened imperilled species is essential to ascertain its adaptation, expansion and opportune reestablishment in natural populations. The study of intra and inter - population genetic variability within and between local populations is profoundly utilizable to gain information on individual identity, breeding patterns, genetic degree of relatedness and genetic diversity/variability within as well as between them. Genetic variations can be assessed by means of DNA polymorphisms.

Molecular markers are realistic and useful tools to for the investigation and monitoring the genetic structure of populations both in native and captive lots condition. RAPD and ISSR techniques are of prime importance for widely used for genetic diversity study because they are easy, cost effective and fast methods, especially when other sophisticated methods are not yet developed. Moreover, the RAPD and ISSR amplifications do not require prior knowledge of flanking sequence of the genome of the concerned species. RAPD-PCR technique has been extensively used to characterize genetic structure as well as to study genetic diversity of different fishes. ISSR primers usually amplify DNA segments present at an amplifiable distance between two oppositely oriented identical microsatellite repeat regions. ISSR markers are generated via PCR reactions amplification with a single

microsatellite primer designed from repetitions of two or three nucleotides anchored (either 5′ or 3′) with in a sequence of one to three nucleotides that aim to eliminate slippage-related artefacts. Some advantages related to these markers are rapidness, requirement of small quantities of template DNA, a fewer/less number of PCR reactions, high PCR reactions annealing temperatures that reduce the quantity of artifacts and multi-locus highly polymorphic marker in nature. Thus, ISSR-PCR technique was preferred many times over to other dominant markers by several groups of researchers in studying genetic diversity at the interspecific and intraspecific levels of species. Therefore, due to the unavailability of microsatellite or SNP markers in *Badis badis* and *Amblyceps mangois* till date, we resorted to the time-tested RAPD and ISSR primers to ascertain and compare the available genetic variations in this ichthyofauna. Moreover, I have analyzed mtCOI gene to observe the genetic and phylogenetic relationship with other geographically isolated population across worldwide; to corroborate the findings based on the RAPD and ISSR markers with that of mtCOI gene

Three major river systems (Mahananda, Teesta and Jaldhaka) have been studied and total seventeen populations (for *Badis badis*) and fourteen (for *Amblyceps mangois*) were selected from different geographic locations for the collection of the fish samples. The catch of the fishes from each location was very low and as the species are belong to the threatened category a non-invasive DNA isolation technique was developed and used for extraction of genomic DNA from the fishes. The genomic DNA was extracted from the minute quantities of fins without sacrificing the organisms. Different diversity indices like total number of polymorphic loci, percentage of polymorphic loci, Nei's genetic diversity, Shannon's information index, and measure of evenness are used to study the genetic architecture of two studied fishes. After compiling all the data and comparing with some other related studies it was found that the genetic diversity of the studied fishes were reasonably positioned within the range of low to moderate level. The mtCOI based analyses also showed that the number of haplotypes and haplotype diversity also low in the studied fishes compared to other related studies. Thus the mtCOI gene based findings also corroborate with that of the RAPD and ISSR based findings. Different level of anthropogenic pressures and several natural processes are lead to the decline of the genetic diversity of the studied ichthyofauna the three river system of the studied region. Although, the Teesta river system populations showed the comparatively greater level of genetic diversity than the other two river system populations viz., Mahananda-Balasan river system and Jaldhaka river system. But the individual population of both the fishes of the Jaldhaka river system showed greater level of diversity

than other river system populations but the Teesta river system showed overall higher level of genetic diversity after considering all the Teesta river populations together. This is happed due to joining of different tributaries to the Teesta river which causes the overall genetic diversity of the Teesta river system population to increase. This situation occurred in the both the studied fishes.

The genetic relationship between different populations of *Badis badis* and *Amblyceps mangois* separately revealed that the three river populations form three distinct groups but the some populations from Mahananda river system showed close relationship with that of the Teesta river system. This is happen due to the connection of Mahananda river system with that of Teesta river system via a narrow channel which leads to the admixture of Mahananda population with that of Teesta river population. Moreover, excessive rain and flood also leads to mixing of river water with nearby tributaries and genetic exchange occurred. This ultimately led to the close genetic and geographic association of the Mahananda and Teesta river system. Whereas the Jaldhaka river system is free from any connection other two river systems so the Jaldhaka river system forms a separate group and the populations showed very close association with each other. It was also found that the pattern of diversity (H´), richness (S) and evenness (E) have varied across all seventeen populations of *Badis badis* and fourteen populations of *Amblyceps mangois* in a substantial manner as the river flows from higher to lower altitude. Several anthropogenic, climatic and geographic factors are responsible for this observed change and alternation of the diversity pattern in the studies fish populations. In my present study a significant levels of genetic divergence and population differentiation occurred in the *Badis badis* and *Amblyceps mangois* populations; and this indicates populations are highly structured as evident by the hierarchical gene diversity analyses. That is, a population having local breeding units in each stream have high genetic divergence from similar breeding units in other streams, and need to be managed as evolutionary significant units (ESU) for conservation purpose. Therefore, in Mahananda river system there is a need to manage the whole river system as evident from the hierarchical analyses to conserve the *Badis badis* and *Amblyceps mangois* population; and in Teesta and Jaldhaka river system it is necessary to manage the first and second order streams to conserve the gene pool of *Badis badis* and *Amblyceps mangois* of the whole river system.

This study is the first attempt to characterize and compare the genetic architecture of *Badis badis* from the three major river system of sub-Himalayan biodiversity hotpost region of West Bengal, India. Low levels of genetic diversity/variation were found in the present

study among the seventeen populations as an indicative of the recent threatened status of this species. In addition to over-fishing, presence of barrage/dam at the upper reaches of the Teesta, pesticide run offs from the nearby tea gardens, and urban effluents could be the possible reasons behind the lower catch frequency and low level of genetic diversity of the studied fish in the Mahananda and Teesta river population. Whereas the Jaldhaka population experiences less anthropogenic pressure leads to comparatively high level of diversity than other two river systems. Therefore, the Jaldhaka population should be managed and conserved to preserve the available gene pool of this threatened species. Information on the available genetic variation and phylogenetic relationship based on the mtCOI gene from the present study should form a baseline on which further studies can be undertaken. As the species is commercially expensive and has an potential ornamental value; and the region being located in the sub-Himalayan biodiversity hotspot region, the management and proper rehabilitation of this threatened ichthyofauna in the river systems is essential from the standpoint of livelihood of rural inhabitants and their socio-economic upliftment.