INTRODUCTION

The fisheries-based blue revolution can become real and sustainable if the production potential of available water resources can be efficiently managed. But there are several areas of concern that need to be addressed to realize this goal. Genetic preservation is essential in order to sustain fishery production, forestry and agriculture (Hilsdorf, 1995). With proper management, a sustainable fishery can continue to provide food and jobs for millions of people around the world without disrupting the ecological systems. But an increase in fishing of natural resources beyond sustainable limits may lead to loss of natural resources and biodiversity (Anonymous, 1999). Over-harvesting coupled with pollution, environmental degradation and habitat fragmentation have contributed to decline or disappearance of fish populations of almost all economically important species. Therefore need for conservation of fish genetics has been recognized by fishery scientists especially in relation to over-fishing of natural stock and effect of large scale alterations to river systems (Banerjee et al., 2008). There is an urgent need of employing proper management strategies to conserve vanishing native fish species. For effective management in conservation biology, knowledge of the genetic structure of natural populations of different fish species is essential. The natural genetic pattern within, between and among populations must be maintained to prevent deletion of indigenous genetic resources. It is important that research programmes be directed towards anticipating emerging opportunities in the field of fisheries while addressing the negative impacts on the ecosystem.

The detection of genetic variations at the species, population and within population level is of great importance for sustainable fisheries. Genetic variations at species level help to identify the taxonomic units and to determine the species distinctiveness. Variations at the population level can provide an idea about different genetic groups, the genetic diversity among them and their evolutionary relationships. The study of genetic variability is of prime importance for genetic approaches to fish conservation or breeding, which depend on knowledge of the amount of variations existing in a local reproductive unit (Carvalho, 1993). Each species must be recognized as a unique case, recognizing the possibility of intra-specific variations in population structure. Very closely related
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Sympatric species can have very different population structures and it is dangerous to generalize from one about the other (Allendorf and Utter, 1979). Biological relationships between populations can be known to the managers by accurate genetic stock identification, which in turn will allow for a more informed management policy for the resource. The information on stock structure of cultivable fish species can be very useful for optimizing identification of stocks, breeding programmes, stock enhancement, management of sustainable yield and preservation of diversity (Shaklee et al., 1990). A substantive knowledge of the genetic structure, stock structure and variability of the population or the species in question, therefore, is needed as the inceptive step prior to putting into effect any conservation or aquaculture attempts (Chong et al., 2000; Nagarajan et al., 2006).

1.1 Bagrid Catfishes

The Siluriformes is one of the richest fish order in number of families and genera and its representatives are commonly known as catfishes. The name catfish is derived from the feelers or barbels that extend from each side of the upper jaw of the fish and in some species, from the lower jaw also, suggesting the similarity to whisker of a cat. The dorsal and pectoral fins are often edged with sharp spines, in some cases poisonous, used in the defense and capable of inflicting severe wounds. In India catfishes belonging to thirteen families have been documented. Most of them are confined to fresh water but some are marine (Talwar and Jhingran, 1991).

Catfishes, particularly of the families Bagridae, Claridae and Pangasidae, are the important aquatic resources in Asia. Bagridae family, comprising of twenty seven genera (six in Indian region) is widely distributed in Asia and Africa. Some species of this family are kept as aquarium fishes, while others are very large and important as food fishes (Talwar and Jhingran, 1991). Catfishes are in great demand in domestic markets, but catfish aquaculture has not yet been developed in India (Tripathi, 1996). These points add value to their research perspectives but little is known about their systematics and biology (including population structure). So this group demands a sound basic platform in the form of its genetic characterization so as to improve its indigenous stocks and for planning management policies and future culturing practices so as to get maximum
benefits in monetary and ecological terms. The genetic characterization of catfishes by means of phenotypic markers, karyotyping, protein and DNA polymorphisms contributes to or forms an integral part of the disciplines of systematics, population genetics, cytogenetics, quantitative genetics, biochemistry, molecular biology and aquaculture (Volckaert and Agnese, 1996).

1.2 Molecular Characterization
DNA polymorphisms have been widely used as a means of assessing genetic diversity in different organisms. The polymorphism between individuals results from the DNA sequence differences between the primer binding sites. Such polymorphism can be detected and used to analyze genetic diversity using a number of DNA markers like restriction fragment length polymorphism (RFLP) (Pogson et al., 1995; Okazaki et al., 1999), microsatellites (Appleyard et al., 2001; Beacham et al., 2002) and randomly amplified polymorphic DNAs - RAPD (Williams et al., 1990; Williams et al., 1993; Lehmann et al., 2000). These markers have several applications in fish genetics and have been extensively used for objectives like determining species-specific markers, population genetic structures and phylogenetic relationships etc. (Uter and Ryman, 1993; Ward and Grewe, 1994; Carvalho and Hauser, 1994). Among these, RAPD technique also called multi-locus “RAPD fingerprinting” (Bagley et al., 2001) has been widely employed as a molecular tool for assessing genetic diversity among populations as well as for studying genetic relationships in number of aquatic species particularly fish (Almeida et al., 2003; Ali et al., 2004; Sandoval-Castellanos et al., 2007). The ease, simplicity and low cost involved in this technique makes it ideal as number of polymorphic markers can be produced easily with no prior knowledge about the genetics of the organism (Welsh and McClelland, 1990; Williams et al., 1990; Fischer et al., 2000; Klinbunga et al., 2000). It is now widely recognized that RAPD reproducible band profiles can be obtained on the agarose gels maintaining consistent reaction conditions and represent an affordable and rapid way to detect polymorphisms directly on gel. Although their dominant nature in diploid individuals is a limiting factor for their use in estimating population genetic parameters (Isabel et al., 1999), and it is not possible to estimate allele frequencies directly but similarity is calculated based on presence or
absence of the marker phenotypes and then these are converted to genetic distances (Chong et al., 2000). Thus RAPD analysis still remains a powerful and inexpensive method to observe genetic variations in different species as well as populations. Perhaps the main reason for the success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA without the requirement for cloning, sequencing or any other form of the molecular characterization of the genome of the species in question. So RAPD fingerprinting offers a rapid and efficient method for generating a new series of genetic markers in fishes (Foo et al., 1995).

1.2.1 Genetic Variation among Mystus Species

RAPD analysis has been used effectively for initial assessment of genetic variations among fish species (Dinesh et al., 1993; Johnson et al., 1994; Foo et al., 1995; Bielawski and Pumo, 1997; Caccone et al., 1997; Cunningham and Mo, 1997; Barman et al., 2002). In spite of great economic and scientific importance, little information is available on the phylogenetic relationships among the catfish species.

Different species of genus Mystus (family Bagridae) have been studied by vast number of authors from different aspects in India and abroad as these are widely distributed in all the South Asian countries. Most of the studies conducted are related to phylogeny (Jayaram, 1971), sexual dimorphism (Swarup and Swaroop, 1975), length-weight relationship (Mirza et al., 1988), reproductive biology (Sudha and Shakuntala, 1989; Wang et al., 1992), haematological studies (Sekar and Christy, 1996), acute toxicity (Rao and Patnaik, 1997; John and Prakash, 1998), immune response (Anbarasu et al., 1998) and feeding relationships (Srivastava et al., 2000). There is lot of confusion relating taxonomy of genus Mystus, though few studies have been reported to resolve the ambiguities (Jayaram, 1955; Jayaram 1971; Roberts, 1994). But molecular analysis has yet not been used considerably in this genus as systematic tool to study phylogeny and species differentiation. Based on considerations that there are no genetic variability studies (characterization, variations and population structure) undertaken on the Mystus species in Indus river system in the country, which are considered to be essential for their effective management, future aquaculture plans and conservation measures, the present
study is an attempt to evaluate genetic relationship among six different species of genus *Mystus* (*M. bleekeri*, *M. cavasius*, *M. vittatus*, *M. tengara*, *M. aor* and *M. seenghala*).

### 1.2.2 Population Variation Studies

Genetic variability is an important characteristic of populations for the short term fitness of individuals as well as for long term survival of the population, permitting adaptation to the changing environmental conditions (Carvalho, 1993). The genetic variability within population is extremely useful to gather the information on individual identity, breeding patterns, degree of relatedness and genetic variations among them (Schierwater et al., 1994). DNA markers are particularly effective tools to analyze population structure and gene flow since variation at the DNA level is considered neutral (Avise, 2004). Among these PCR based multi-locus DNA fingerprints represent one of the most informative and cost effective measures of genetic diversity and are useful population level biomarkers. RAPD analysis has been extensively used to analyze genetic variability in different fish populations (Dergam et al., 1998; Dergam et al., 2002; Nagarajan et al., 2006; Muneer et al., 2008).

Among the studied Bagrid species, the giant river catfish, *Mystus seenghala* (Sykes) also known as *Aorichthys seenghala* as well as *Sperata seenghala* is one of the common species of fishes of the family Bagridae found in South Asia. This species is distributed in India, Pakistan, Bangladesh, Afghanistan and Nepal (Talwar and Jhingran, 1991; Jayaram, 2002) and is easily recognized by broad, spatulate snout with smooth upper surface, brownish-grey back, silvery flanks and belly and a dark well-defined spot on the adipose dorsal fin. It is mainly a riverine fish but is also inhabiting a few other freshwater habitats (Talwar and Jhingran, 1991). It is predatory in habits attacking small carps, prawns and is relished for its good taste (Chandy, 1994). *M. seenghala* is the most preferred fish species in north and north-western states of India due to its tasty flesh and fewer intramuscular bones. It has been one of the important species of catfishes to capture as it fetches higher price than carps (Tripathi, 1996). Inspite of its great demand in Indian domestic market this species has yet not been explored for its aquaculture potential (Tripathi, 1996). The entire demand of this fish in domestic market is met
through capture from river bodies, thus effective management of wild stocks is critical. Scientifically sound management of fish resources depends on the basic knowledge of the biology of the species, including information on population structure. This kind of information is useful for the development of management strategies that will conserve biodiversity associated with different species, sub-species, stocks and races (Turan et al., 2005). So a detailed knowledge on population structure of this fish is needed for sound management of its resources as well as to exploit it commercially.

RAPD analysis has provided important applications in population variability studies of catfishes. Chong et al. (2000) represented the first application of the RAPD technique and amplified fragment length polymorphism (AFLP) technique in the study of genetic variations within and among five geographical populations of Malaysian river catfish (*Mystus nemurus*). Similarly, RAPD-PCR was used to examine genetic similarity and diversity of cultured catfish (*Silurus asotus*) populations collected from two areas in western Korea by Yoon and Kim (2001) and significant genetic differentiation between the two populations was found on the basis of RAPD analysis. Muneer et al. (2008) confirmed the suitability of RAPD markers for the study of population genetic structure in yellow catfish *Horabagrus brachysoma* stocks. Therefore this study was also aimed to assess the level of genetic variability within and between the populations of giant river catfish *M. seenghala* in Indus river system using RAPD markers.

### 1.3 Morphometric Characterization

Morphometric analysis has been very useful for separating species, populations and races and also widely used for the identification of stocks of fish (Teugles, 1982; Johal et al., 1994; Singh and Singh, 2000; Tiwari and Qureshi, 2003; Usha and Prakasam, 2003; Turan et al., 2004, Turan et al., 2005; Johal and Kaur, 2005). But for the characterization of species and populations morphometric methods alone are not sufficient. The process is restricted to phenotypic characters only and moreover it does not give detailed estimate of genetic variability. All these limitations can be overcome by supporting the molecular data by morphometric comparisons and analysis. The data can be used to estimate the amount of variability within and among the species and also the population structure of a particular species. Correlations between genetic variations and morphological variations
have been confirmed by workers in natural populations (Soule and Zegers, 1996; Poulet et al., 2004) and both have been used to make similar assessment of population differentiation (Sugg et al., 1997).

Morphometric studies of fish population are very important for understanding the interactive effect of environment, selection and heredity on the body shapes and sizes within a species (Cadrin, 2000). Morphometric variations between stocks can provide a basis for stock structure and might be more applicable for studying a short-term, environmentally induced variations (Begg et al., 1999). Environment and genome are the two interacting processes which act on the developmental program for forming a phenotype (Scheiner, 1993). Genetic variations for a fixed phenotype have been hypothesized to be favored in stable environments (Hori, 1993), whereas phenotypic plasticity can be an important adaptive strategy for coping with environmental variability (Stearns, 1989 and Scheiner, 1993) and the knowledge of exact plastic (environmentally controlled) and nonplastic (genetically controlled) characters is essential for identification of species as well as populations of a species (Johal and Kaur, 2005). Therefore, knowledge of extent of phenotypic plasticity in a population of a particular fish species can be of immense importance. In this study morphometric variations within the populations of *M. seenghala* from river Sutlej and Beas were statistically analyzed to categorize genetically controlled and environmentally controlled characters.

Several studies on the comparative morphometrics of different fish populations have been conducted (Nakamura 2003; Turan et al., 2005; Ibanez-Aguirre et al., 2006). Animals with the same morphometric characteristics are often assumed to constitute a stock; this idea has been used widely in fishery stock differentiation studies (Avsar, 1994). So, the morphometric characteristics of *M. seenghala* populations from rivers Sutlej and Beas were also compared to determine the proportion of influence of genetic factors on the observed morphometric characters. The objective behind this was to use statistical techniques to analyze and compare the size-free shape of different geographic populations of this fish in two rivers of Indus river system to elucidate morphometric variations in this species.
1.4 **Indus River System**
Fisheries professionals have set their eyes on the Indus river system resources to give impetus to the “blue revolution” in the country. The Indian Society of Fisheries Professional, Mumbai, has observed that aquaculture, based on indigenous and judiciously selected exotic fish species particularly catfishes available in the Indus river system, has the potential as much to tickle the palate and to fill pockets of fish farmers with cash (Anonymous, 2005). The left bank tributaries of the Indus river system, i.e., Jhelum, the Chenab, the Ravi, the Beas and the Sutlej with a combined length of 5,000 km have been identified for exploration and exploitation of the “blue” wealth (Sehgal, 2005). These rivers have been found to abound in as many as 85 species of fish, which are an important component of region’s biodiversity and are a valuable genetic resource for the future. Unfortunately, these fishes are threatened due to environmental degradation and overexploitation. There is an urgent need for a consolidated effort to promote their conservation and to develop and implement effective management strategies for maintaining healthy fish stocks. Keeping in view the immense importance, the Indian tributaries of Indus river system i.e. rivers Sutlej, Beas and Ravi, have been selected for the present study.

1.5 **Objectives**
The present work has been carried out with following objectives:

- To determine the genetic variation within and between different populations of the fish *Mystus seenghala* using molecular markers (RAPD).
- To study the morphological variations in different populations of the fish *Mystus seenghala* using morphometric characters and meristic counts.

The outcome of this study will provide RAPD markers and morphometric comparisons to be utilized in genetic programmes and for planning conservation as well as management of natural resources of these economically important catfish species.