REVIEW OF LITERATURE

Genetic variation can be thought of as the ‘‘fuel’’ for evolution, as the process of continued evolution is critically dependent on renewed variation. From an evolutionary standpoint the progressive accumulation of genetic variation is thought to have given rise, beginning with common ancestors, to the diversity of life. Several evolutionary forces affect the amount and distribution of genetic variation among populations and thereby population differentiation (Felsenstein, 1985). Geographic distance and physical barriers enhance reproductive isolation by limiting the migration and increased genetic differentiation between populations (Allendorf and Ryman, 2002).

Genetic characterization of catfishes by means of phenotypic markers, karyotyping, protein and DNA polymorphisms contributes to the disciplines of systematics, population genetics, cytogenetics, quantitative genetics, biochemistry, molecular biology and aquaculture (Volckaert and Agnese, 1996). Perusal of the literature reveals that the general approach to research is pragmatic. The Bagridae do not include model species for fundamental genetic research. The Clariidae and the Ictaluridae represent the best studied families. The systematic status of a number of species and families has been either elucidated or confirmed by genetic approaches. The genetic structure as well as gene flow among natural populations has been documented in relatively few cases, while the evaluation of strains of catfishes for aquaculture (especially Ictaluridae and Clariidae) is in progress (Tripathi, 1996). It appears that a more detailed knowledge of catfish populations is required from two perspectives, first for natural populations, which are threatened by habitat loss and are poorly characterized at the genetic level and secondly for the selection of suitable strains for aquaculture. Implementation should pose no problems, as the present powerful means such as DNA characterization combined with protein polymorphisms can resolve the above mentioned issues (Volckaert and Agnese, 1996). Increased computational power and mathematical models have enhanced the scope of conclusions that can be drawn out of the data generated. Molecular markers provide direct assessment of pattern and distribution of genetic variations (Ferguson et al., 1995). The studies of morphometric and meristic characteristics, are being completed
Review of Literature

by the data from new molecular techniques (Beardmore et al., 1997). The use of molecular genetic markers alongside traditional taxonomic methods can help to clarify species relationships (Miya and Nishida, 1997).

Bagrid catfishes of genus Mystus have been studied by a number of workers in India and abroad from different aspects (Bhatt, 1972; Dhaliwal, 1975; Desai and Rao, 1972; Swarup and Swaroop, 1975; Bhatt et al., 1977; Sudha and Shakuntala, 1989; Wang and Luo, 1992; Wang et al., 1992; Rajyalakshmi, 1996; Sekar and Christy, 1996; Rao and Patnaik, 1997; John and Prakash, 1998; Anbarasu et al., 1998; Srivastava et al., 2000).

Set al et al. (1998) performed a population study based on morphological characters of the fish A. seenghala (Sykes) to identify its different races from the rivers, Ganga and Yamuna, having different ecological conditions. The investigation revealed no marked morphological variation in the sampled specimen, indicating an unit stock. Jayaram (1971) elucidated the distinguishing osteological features of catfishes of the subgenus Aorichthys of genus Mystus and discussed their phyletic significance. Aorichthys is unique in possessing an interneural shield in between the basal bone of the dorsal fin and occipital process. Further, the cranium is more compact than in Mystus, and the inferior limb of the post-temporal is deeply excavated on the posterior side, which are specialized features. These indicate its relationship with some African genera, such as Porcus Geoffroy St. Hilaire. To reflect the true phylogeny of these fishes, Aorichthys is raised to the rank of a genus having two species. Roberts (1992) did revision of the striped catfishes of Thailand, misidentified as M. vittatus, with descriptions of two new species (Pisces: Bagridae). Boldly striped species of the Asian bagrid catfish genus Mystus are common in lowland habitats of the Mekong, Chao Phraya and Meklong basins of Thailand. He recognized only a single species, M. vittatus. This species, however, is restricted to the Indian subcontinent. Tripathi (1996) described A. aor, A. seenghala, M. cavasius, M. gulio, Rita rita, Wallago attu, Ompok immaculate, Heteropeustes fossilis, Clarias batrachus, Silonia silonia and Pangasias pangasius as the commercially important fish which contribute substantially to the total inland fish production in South Asia. He discussed that in comparison to carp culture, catfish culture has just begun in the region. Techniques of seed production have been standardized in C. batrachus, H. fossilis.
and *W. attu* which can be spawned even repeatedly at short intervals of 30-40 days. Such methods are, however, in an experimental stage for other species. *C. batrachus* is the most popular and its traditional culture in rice fields is well known. The catfish species fetch a very high price in India and Bangladesh and has a great export potential. He opined that a vast grow-out potential exists all over the region for small catfish in carp nurseries as a second crop. Extensive culture of large catfish in derelict ponds could be an intermediate step in their improvement through control of all trash fish. With increased seed availability, semi-intensive and intensive mono-culture systems, being tried experimentally could be developed in the view of an industrial production of catfishes as new export items from the region, if conditions for proper water quality management, aeration and partial replenishment are maintained.

Seth and Katiha (2001) studied the riverine fisheries of large sized siluroids with special reference to *A. seenghala* (Sykes). The significance of large siluroids in dynamics of riverine fish catch composition pinpointed the need to appraise its status in riverine fisheries and the factors influencing their fisheries with special reference to *A. seenghala* (Sykes). The study concluded that, although, catch of large siluroids had declined but their share in riverine catch increased. The same was true for *A. seenghala*. The increase in fishing intensity in *A. seenghala* breeding grounds was one of the evident indicators of fishing stress on the fish. But none of the studies are directed towards the genetic characterization of *Mystus* species in Indus river system, therefore present study is an attempt to fill this gap and can be further utilized for the effective management, future aquaculture plans and conservation measures of these species in this river system.

### 2.1 Molecular Characterization Studies

The advances in DNA techniques have a great impact in addressing problems in many aspects of biology. Molecular markers have totally changed our view of nature in less than half a century and in the process they have evolved themselves. Within the last decade, advancement in technology has increasingly supported the use of genetics in determining population diversity. Many molecular techniques are now available, which allow ecologists and evolutionary biologists to determine the genetic architecture of a wide variety of closely related individuals. Molecular markers are classified into two
categories: type I are markers associated with genes of known function, while type II markers are associated with anonymous genomic segments (O’Brien, 1991). Type I markers have utility in studies of comparative genomics, genome evolution, candidate gene identification etc. and also serve as a bridge for comparison and transfer of genomic information from a map-rich species into a relatively map-poor species. In general, type II markers such as RAPDs, microsatellites, and amplified fragment length polymorphisms (AFLPs) are considered to be non-coding and therefore selectively neutral. Such markers have found widespread use in population genetic studies, where characterizations of genetic diversity and divergence within and among populations are based on assumptions of Hardy-Weinberg equilibrium and selective neutrality of the markers employed (Brown and Epifanio, 2003).

Molecular markers have a considerable potential to fisheries management (Utter, 1991). These genetic tools can be used for management and conservation of fish species and can be applied to three areas in fisheries: Stock culture analysis, Aquaculture and Taxonomy/systematics (Ward and Grewe, 1994). The study of fish populations using molecular markers started some 50-55 years back. While many genetic studies of fish populations have been done for improved management of this important natural food and recreational resource, it is being appreciated increasingly that variation within and among all fish species is an important component of biodiversity and as such should be conserved for its intrinsic value (Ferguson et al., 1995). Genetic variants can be used as markers in different studies of natural and cultured fish populations. These molecular genetic markers are of two types: Protein and DNA. The development of starch gel electrophoresis (Smithies, 1955), along with histochemical staining (Hunter and Markert, 1957) made the detection of enzyme/protein polymorphisms possible and this provided first simple genetic markers for studying natural populations. Earlier studies used blood group polymorphisms to discriminate between spatially discrete populations of fish (Sick, 1961), but the patterns observed were difficult to interpret. Due to this problem, specific histochemical stain procedures were included in the research (Hunter and Markert, 1957). Specific proteins were stained after starch gel electrophoresis which allowed the detection of allozyme variation (Harris and Hopkinson, 1976). Electrophoretic
investigations were chosen enthusiastically by fishery scientists to investigate population structure (Moller, 1970; Avise and Smith, 1974). Initial studies in 1960’s involved proteins as markers (allozyme variation), it provided a fast and comparatively inexpensive method to the scientists for measuring genetic variability at protein level in natural populations. From 1964 onwards, fish population studies are being done using electrophoretic analysis of protein variants (Utter, 1991). In next 2-3 decades the patterns of allozyme variation were characterized in many species. Allendorf et al. (1976) did an electrophoretic examination to understand the genetic structure of Scandinavian brown trout (Salmo trutta L.) in the different lakes of northern Sweden. Genetically distinct and reproductively isolated populations of brown trout, Salmo trutta L. from Lough Melvin Ireland were evidenced using electrophoretic studies of five polymorphic enzyme loci by Ferguson and Mason (1981). Stoneking et al. (1981) did starch gel electrophoresis for 39 loci to reveal the genetic differences between northern and southern populations of Brook trout (Salvelinus fontinalis). Carlson et al. (1982) revealed low genetic variability in Paddlefish, Polyodon spathula populations in north America, using starch gel electrophoresis. Buth and Crabtree (1982) examined genetic variability and population structure of Catostomus santaanae in Santa Clara drainage in southern California electrophoretically for the gene products of 33 presumptive loci. Grant (1984) described the genetic population structure of Atlantic herring, Clupea harengus using electrophoretically-detectable protein variants. Grant and Stahl (1988) observed genetic variation in Atlantic cod, Gadus morhua and Pacific cod, Gadus macrocephalus using electrophoretic analysis.

In 1980s, the workers turned to direct investigation of DNA sequence to increase the number of variable genetic markers. Initially main focus was on mitochondrial DNA using Restriction fragment length polymorphism (RFLP) analysis (Lansman et al., 1981), Later on Randomly Amplified Polymorphic DNA (RAPDs) (Welsh and McClelland, 1990; Williams et al., 1990) and RFLP analysis of nuclear DNA (Pogson et al., 1995) have also been widely used in fisheries. However recently more attention have been paid on the use of variable number of tandem repeat loci, VNTRS (Jeffreys et al., 1985a,b) which include short tandemly repeated sequences of DNA, randomly distributed
throughout the genome. Initially the main focus was on multilocus DNA fingerprinting, which gave complex band patterns unique for each individual. Difficulties in between gel comparisons and in interpretations emphasized the use of single locus VNTR markers. Now a days most of the research is focused on a second class of VNTR loci, microsatellites that are widely applied in different wildlife and captive management applications. Microsatellites are being used because of a very high levels of variability observed in them.

Though these days, Randomly Amplified Polymorphic DNA is being superseded by other DNA markers (such as microsatellites or SNPs). But RAPD analysis still remains a powerful and inexpensive method to observe genetic variation in different species. 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.

### 2.1.1 Principle of RAPD Technique

The standard RAPD technology (Williams et al., 1990) utilises short synthetic oligonucleotides (10 bp long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR. Amplification products are generally separated on agarose gels and stained with ethidium bromide. Decamer primers are commercially available from various sources. At an appropriate annealing temperature during the thermal cycle, oligonucleotide primers of random sequence bind several priming sites on the complementary sequences in the template genomic DNA and produce discrete DNA products if these priming sites are within an amplifiable distance of each other. The profile of amplified DNA primarily depends on nucleotide sequence homology between the template DNA and oligonucleotide primer at the end of each amplified product. Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites. Sequence characterized amplified regions (SCARs) analysis of RAPD polymorphisms (Paran and Michelmore, 1993; Bardakci and Skinbinski, 1999) showed
that one cause of RAPD polymorphisms is chromosomal rearrangements such as insertions/deletions. Therefore, amplification products from the same alleles in a heterozygote differ in length and will be detected as presence or absence of bands in the RAPD profile. The profile of RAPD bands is similar to that of low stringency minisatellite DNA fingerprinting patterns and is therefore also termed RAPD fingerprinting. On average, each primer directs amplification of several discrete loci in the genome so that allelism is not distinguishable in RAPD patterns. In other words, it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous or homozygous. RAPD markers are therefore multilocus, dominant markers.

2.1.2 Applications of RAPD Analysis

RAPD technique has found a wide range of applications in many areas of biology, because of its simplicity and low cost. Some of the areas where the technique is used are described below:

2.1.2.1 Genetic Variability Studies

The RAPD-PCR method can be applied to detect genetic diversity and similarity in numerous organisms using various primers (Welsh et al., 1991; Levin et al., 1993; Cagigas et al., 1999; Bernardi and Talley, 2000). These have been extensively employed across various taxonomic groups, e.g., Fungi: Muller et al., 2005; Plants: Coletto Filho et al., 2000; Hilfiker et al., 2004; Tang et al., 2006; Arslam and Okumus, 2006; Aphids: Black et al., 1992; Bees: Lu and Rank, 1996; Birds: Haig et al., 2001; Marsupials: Fowler et al., 1998. The results show that the RAPD assay is a powerful approach for identifying genetic and geographic polymorphism. 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 of toxicological and other anthropogenic impacts (Bagley et al., 2001).

The study of genetic variability is of prime importance for genetic approaches to fish conservation or breeding, which depends on knowledge of the amount of variation existing in a local reproductive unit (Carvalho, 1993). RAPD technique is also applied in large number of fish species to detect genetic variability and a few are cited here:
Bielawski and Pumo (1997); Yoon and Kim (2001); Yue et al. (2002); Cavallari et al. (2006).

2.1.2.2 Genetic Mapping
The prior knowledge of sequence needed to design specific primers is a limiting factor in developing large numbers of genetic markers for many organisms (Goodier and Davidson, 1993). The discovery that PCR with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers for a variety of purposes (Williams et al., 1990). The ease and simplicity of RAPD technique makes it ideal for genetic mapping. Genetic mapping has commonly been done using RFLPs (Botstein et al., 1980). But RFLP analysis is time consuming and the identification and isolation of clones is often tedious, so there is increasing interest in PCR technology, which allows amplification of any sequence of interest from nanogram amounts of DNA, and direct visualization of the amplified product. Although necessity for sequence information for PCR was circumvented using short primers of arbitrary sequences to amplify DNA segments, namely RAPD. The speed and efficiency of RAPD analysis encouraged scientists to perform high-density genetic mapping in many plant species such as alfalfa (Kiss et al., 1993), faba bean (Toress et al., 1993) and apple (Hemmat et al., 1994) in a relatively short time. The RAPD approach has also been widely used to create genetic maps in fish species. Postlethwait et al. (1994) mapped 401 polymorphic DNA markers in zebrafish (Danio rerio). RAPD markers are also being used to construct genetic maps of tilapia species, O. niloticus and O. aureus (Naish et al., 1995) and rainbow trout, Oncorhynchus mykiss (Jackson et al., 1995). One disadvantage of RAPD markers is that they are dominant, hence the statistical information generated is less per marker in F2 populations. Therefore, when mapping with dominant markers, it is necessary to use backcross or recombinant inbred populations, haploid or gametophytic tissue, or alternatively an F2 population where only RAPD markers amplified from a single parent are mapped (Williams et al., 1993).

2.1.2.3 Genetic Markers Linked to a Trait in Question
RAPD technique can be widely used in the identification of markers linked to traits of interest without the necessity for mapping the entire genome. Martin et al. (1991) have
described an efficient method based on the RAPD technique to isolate DNA segments linked to certain traits. This approach based on near-isogenic lines (NILs) is accomplished by repeatedly backcrossing a line carrying a gene of interest (donor parent) to a cultivated line having otherwise desirable characteristics (recurrent parent). The introgression of the target gene produces a line with a small segment of donor parent genome in a genetic background, which is almost exclusively from the recurrent parent. Thus, markers that show polymorphisms between these two lines are likely to be linked to the gene of interest. RAPD analysis of NILs has been successful in identifying markers linked to disease resistance genes in tomato \( \text{(Lycopersicon sp.)} \) (Martin et al., 1991), in lettuce \( \text{(Lactuca sp.)} \) (Paran et al., 1991) and in common bean \( \text{(Phaseolus vulgaris)} \) (Adan-Blondan et al., 1994). Klein-Lankhorst et al. (1991) identified chromosome specific RAPD markers in tomato. Kovacs et al. (2000) searched for sex-specific DNA sequences in the male and female genomes of African catfish \( \text{Clarias gariepinus} \) by comparative RAPD assays performed on pooled DNA samples. Two sex-linked RAPD markers were identified from the male DNA pool and confirmed on individual samples, showing good agreement with phenotypic sex. This allowed for rapid, molecular sexing of the species on the basis of a simple three band (male) versus one band (female) pattern. These are the first sex-specific DNA markers isolated from a siluroid fish species.

**2.1.2.4 Population and Evolutionary Genetics**

RAPDs may provide insights into organismal evolution that are overlooked by single-gene comparisons. Combining RAPD-PCR and sequencing methods to produce phylogenetic characters thus still may hold some promise in evolutionary genetics and systematics. Determining the nucleotide sequences of randomly amplified products found in homologies between RAPDs could add inference of relationship with greater confidence which could be used as phylogenetic characters. Such an approach was explored in cichlids by Sultmann et al. (1995), who showed that the RAPD-PCR technique followed by sequencing of selected fragments produced phylogenetic characters.

The utility of these markers for detection of population differences agrees with previous
reports in a diversity of other organisms: a wild yam species, *Dioscorea tokoro* (Terauchi and Konuma, 1994); different populations of Mediterranean fruit flies, *Ceratitis capitata* (Baruffi et al., 1995), the malarial mosquito, *Anopheles gambiae* (Lehmann et al., 1996), Atlantic salmon, *Salmo salar* L. (Sanchez et al., 1996), and Japanese native breeds of chicken (Takahishi et al., 1998).

Sandoval-Castellanos et al. (2007) analyzed RAPD data generated from 210 samples of jumbo squid *Dosidicus gigas* from eight eastern pacific sites to evaluate genetic structure and to measure the impact of temporal variation. And to determine genetic differentiation between populations in order to define the structure of stock populations of *D. gigas*. These markers are being used in number of plant species also. Marillia and Scolles (1996) used RAPD markers in *Hordeum* phylogeny. The phylogenetic relationships among 39 wild *Hordeum* species, subspecies, and cultivated barley were investigated using RAPD markers as discriminating characters. The results demonstrated that RAPD technology represents a useful and reliable tool for detecting polymorphism for phylogenetic studies. Similarly, a study by Khan and Narayan (2007) confirmed that the RAPD analysis is suitable for studying phylogenetic relationships between related species. They used fifty six *Nicotiana* species to construct phylogenetic trees and to assess the genetic relationships between them.

### 2.1.2.5 Plant and Animal Breeding

Genetic improvement of animals is limited by the fact that most traits of economic importance are polygenic in nature and are influenced by a variety of external (environmental) and internal factors. Such traits are termed quantitative traits and polygenic loci involved in their expression are termed quantitative trait loci (QTL). To date, RFLP markers have been used as genetic markers to monitor the transmission of useful QTL alleles from generation to generation in the course of breeding programmes (Beckmann and Soller, 1983; Soller and Backmann, 1983). As stated above, the RAPD technique enabled the development of large numbers of genetic markers more efficiently than RFLP based methods that have been used to construct maps of complex genomes. These markers can be used in monitoring these loci during introgression and selection programmes. Markers linked to simple commercially important genetic traits such as
disease resistance genes can also be identified from natural resources and introgressed into domestic strains or varieties. The ability of the RAPD technique to reveal intra-specific variation can be used in screening for the degree of inbreeding in commercial plant and animal species to prevent an increase in the frequency of deleterious recessive alleles in populations. Polymorphic RAPD markers transformed to SCAR markers can be more advantageous in commercial breeding programmes if a quick plus/minus assay can be developed to detect the presence /absence of the product (Paran and Michelmore, 1993). Genetic and geographic polymorphism of cultivated tobaccos (*Nicotiana tabacum*) in Turkey were studied using RAPD by Arslan and Okumus (2006). RAPD technique was used to evaluate both genetic diversity among 21 primitive tobacco accessions comparing flue cure virginia genotype (FCV) and their geographical polymorphism as a source of genetic variations for breeding programmes.

2.1.3 Advantages and Disadvantages of RAPDs

The RAPD technique has an advantage over other systems of genetic documentation because it uses universal sets of primers, and no preliminary work such as probe isolation, filter preparation, or nucleotide sequencing is necessary. These primers adhere to a specific nucleotide segment of the genomic DNA. The DNA is cut into many segments of a specific length which can be measured using gel electrophoresis. For a mutation to change the RAPD pattern, it must occur in the priming region or must change the length of the DNA between priming regions. In this way the RAPD analysis can provide a simple and reliable method for measuring genomic variation. Because the RAPD approach is a relatively straight-forward technique to apply, and the number of loci that can be examined is unlimited, RAPD analysis is viewed as having a number of advantages over RFLP’s and other techniques (Lynch and Milligan, 1994). In many instance, only a small number of primers are necessary to identify polymorphism within species. The ease of the RAPD technique could lead to the automation of genetic mapping and to the extension of genetic analysis to cover organisms which lack an ample number of phenotypic markers to completely describe their genome (Williams et al., 1990). For any population a selective process can produce change only if there is variation to select among. Genetic differences within populations were easily detectable.
using RAPD analyses with single-primer DNA amplifications (Mulcahy et al., 1993; Mulcahy et al. 1995; Vicario et al., 1995). RAPD methods showed a more pronounced effect of isolation-by-distance in comparison with allozymes (Mamuris et al., 1999). Additional findings supported the use of RAPD analysis as an effective tool in species identification and cross-contamination test among different cell lines (Gao et al., 2001). The RAPD-PCR method can be applied to detect genetic diversity and similarity in numerous organisms using various primers (Welsh et al., 1991; Levin et al., 1993; Cagigas et al., 1999; Bernardi and Talley, 2000). For all of these reasons, the RAPD assay has been used to construct phylogenetic trees for resolving taxonomic problems in many organisms (Chalmers et al., 1992; Bardakci and Skibinski, 1994; Greef and Triest, 1999). It has been concluded that although AFLP analysis is superior in terms of efficiency, RAPDs may still be used as reliable markers in small low-tech laboratories (Kjolner et al., 2004). Some limitations restrict practical application of RAPD analysis (e.g. dominance, reproducibility, homology inferences and artifact fragments). Dominance is a major limitation of the RAPD approach. RAPD markers are thought to be dominant, with polymorphisms detected as either band presence or absence. Dominant markers are not as efficient as co-dominant markers for population genetics studies (Lewis and Snow, 1992; Lynch and Milligan, 1994). Lynch and Milligan (1994) estimated that 2–10 times more individuals need to be sampled per locus for dominant markers compared to co-dominant markers. Krauss and Peakall (1998) suggested that this disadvantage may be overwhelmed because of the large number of available polymorphisms, typically over 100 polymorphisms per gel-lane are possible. Concerns about reproducibility of RAPDs have limited their wider use in environmental biology. Several studies have reported poor reproducibility for RAPD markers (Penner et al., 1993; Skroch and Nienhuis, 1995). Bagley et al. (2001) assessed polymorphism and reproducibility of the two common fingerprinting techniques, RAPD and AFLP in pedigreed populations of rainbow trout (O. mykiss) to derive general rules for selective removal of problematic fingerprint bands. They found that by excluding bands that comprised less than 1% of total intensity, and by excluding the largest and smallest 10% of the bands, they could achieve nearly 100% reproducibility of AFLP fingerprints. Similar application of band exclusion criteria to RAPD fingerprints did not significantly
enhance their reproducibility, and on average at least 15% of RAPD bands were not fully repeatable, heritable, or transmittable. More generally, the use of RAPDs as systematic characters has several limitations, and the relevance and taxonomic meaning of RAPD groupings requires careful comparison with results of other sources of data (Hseu et al., 1996). It has been documented that once optimized, the RAPD technique is a rapid, reproducible, and powerful method for identification of bacterial (Legionella) isolates to the species level without further restriction or hybridization (Presti et al., 1998). To ensure that amplified DNA bands derive from genomic DNA, and not primer artifacts, negative control should be run for each primer/breed combination (Ali, 2003). Despite all of these limitations (dominance and low reproducibility due to low stringent PCR), RAPD analysis has been used effectively for initial assessment of genetic variation 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).

2.1.4 Species-Specificity and Phylogenetic Studies of Fishes using RAPD

RAPD data has been used for phylogenetic studies and generally supported existing taxonomies based on morphology, isozymes and RFLPs. The studies on the utility of RAPD markers in the phylogeny of cichlid fishes (Sultmann et al., 1995) supported the classical hypotheses of their phylogenetic relationships. The technique has also been used to study genetic variation in several fish species. Bardakci and Skibinski (1994) and Naish et al. (1995) used RAPD markers to discriminate between commercially important tilapia species, subspecies and strains of tilapia. RAPD markers were also generated for several tropical fish species representing seven families (Dinesh et al., 1993). Furthermore, RAPD analysis revealed high levels of genetic variation among individuals from the same broodstock of sea bass (Dicentrarchus labrax) (Alegrucci et al., 1995). Although the value of RAPD markers in taxonomic and phylogenetic studies is not very clear, there is no doubt that these markers can be used for diagnostic purposes. RAPD markers unique to individuals from one species within a genus will be species-specific (inter-specific). Similarly, genus-specific markers can be generated if the fragment is a unique polymorphism to individuals belonging to a certain genus. Species-specific markers can be used in inter-specific gene flow and hybrid identification. Similarly,
population-specific markers will be useful in identification of hybrid populations (Hadrys et al., 1992).

The RAPD technique was investigated as a potential fish species identification tool among eight species of fish- Barramundi (*Lates calcarifer*), Nile perch (*Lates niloticus*), John dory (*Zeus faber*), Mirror dory (*Zenopsis nebulosa*), Silver dory (*Cyttus australis*), Spikey oreo (*Neocyttus rhomboidalis*), Warty oreo (*Allocyttus verrucosus*) and Smooth oreo (*Pseudocyttus maculatus*) (Partis and Wells, 1996). The results showed that RAPD profiles were consistent within this group. Bardakci and Skibinski (1994) used RAPD analysis to discriminate among three species of the tilapia genus *Oreochromis* and four subspecies of *O. niloticus*. Different RAPD fragment patterns were observed for different species, although not always for different subspecies. Evidence was presented that RAPD markers might be useful for systemic investigations at the level of species and subspecies (Bardakci and Skibinski, 1994). Atlantic coastal striped bass (*Morone saxatilis*) exhibit exceptionally low levels of genetic variation. The ability of RAPDs to reveal genetic variation in this highly conserved species has been investigated (Bielawski and Pumo, 1997).

Chiari and Sodre (2001) applied RAPD techniques to eight Anostomidae species to analyze the genetic variability and genetic similarity within and between these species. Similarly, Borowsky and Vidthayanon (2001) assessed the genetic variabilities in four cave and eight surface species of balitorid freshwater fishes from Thailand using RAPD assessments. Cave species have consistently lower RAPD variation than surface species and it is hypothesized that this difference is a function of reduced population size in cave fishes. Barman et al. (2002) evaluated the use of the RAPD assay as a source of genetic markers to generate species-specific RAPD profiles for four species of Indian carps and to estimate genetic variation among these four species. The authors demonstrated that kalbasu is the closest to rohu and the farthest from mrigal. To estimate the genetic diversity in Asian arowana (*Scleropages formosus*) by genotyping fish individuals from three different sources (Yue et al., 2002), three DNA marker systems (RAPD, AFLP and microsatellites) were used.
Review of Literature

Comparative analysis of randomly amplified polymorphic DNA (RAPD) profiles of six Labeo species viz., *L. bata* (bata), *L. calbasu* (calbasu), *L. dyocheilus* (dyocheilus), *L. fimbriatus* (fimbriatus), *L. gonius* (gonius) and *L. rohita* (rohu) at the nuclear DNA variation level (Das *et al.*, 2005), provided evidence that RAPD could be used for genetic differentiation of closely related species, proving RAPD to be an effective technique for the assessment of genetic variation and differentiation at the species level. Similarly, Mohindra *et al.* (2007) investigated genetic relatedness between five species of Mahseer group (*Tor putitora*, *T. tor*, *T. khudree*, *T. mosal mahanadicus* and *Neolissochilus hexagonolepis*) from Indian peninsula using RAPD analysis. This study also revealed that combination of RAPD markers could discriminate the species.

In spite of enormous significance of catfishes little data is available on the phylogenetic relationships among the catfish species and populations. RAPD technique is one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (Bartish *et al.*, 2000) and has provided important applications in catfishes.

The channel catfish (*Ictalurus punctatus*) is the most important cultured fish in the United States of America, accounting for over 50% of all aquaculture production. It is also one of the important sporting fish in many southern states. Liu *et al.* (1998) studied the segregation of RAPD markers in F1 hybrids and backcross hybrids in this species to evaluate the feasibility of using RAPD markers for both intraspecific mating plans and site-specific hybrid mating plans. An additional 100 RAPD primers were evaluated for their usefulness in catfish. Of the 100 primers, 42 were good; 33 were of medium quality; and 25 were poor (Liu *et al.*, 1999). A total of 462 new polymorphic RAPD markers were identified in this study. One hundred RAPD primers have been used to identify DNA-based genetic polymorphism for constructing a genetic linkage map of catfish. Overall polymorphism was low among strains within a species for both channel catfish and blue catfish (*Ictalurus furcatus*). However, considerably higher levels of polymorphism were detected between channel catfish and blue catfish (Liu *et al.*, 1999).

Callejas and Ochando (2001) identified the eight species of genus *Barbus* (Cyprinidae) of
the Iberian Peninsula, whose morphological differentiation is difficult, using RAPD analysis. Different RAPD profiles were observed for the different species. Four species-specific markers were found in *B. bocagei*, seven in *B. comizo*, five in *B. graellsii*, three in *B. guiraonis*, eight in *B. haasi*, thirteen in *B. meriodionalis*, four in *B. microcephalus* and four in *B. sclateri*. Evidence is presented that RAPD markers constitute useful tools for accurate taxonomic identification of Spanish barbels which is one of the first prerequisites in effective conservation programmes. This study indicated that the RAPD technique, despite its reproducibility problem, provides a reliable and useful tool for the identification of *Barbus* species. A set of RAPD markers that unambiguously differentiated taxa was identified and can be used to characterize *Barbus* populations.

Callejas and Ochando (2002) used RAPD markers to estimate the population structure and phylogenetic relationships among eight species of the genus *Barbus* (Pisces, Cyprinidae) that inhabit the Iberian Peninsula. A total of 270 markers were detected that revealed low levels of genetic variability. Results demonstrated RAPD as an advantageous and useful tool for studying the genetic structure and phylogenetics of the species and population levels of *Barbus*.

### 2.1.5 Population Variation Studies of Fishes using RAPD

Limited data is available on the genetic background of the natural populations. Similar species found in different geographical areas need not necessarily have the same genetic constitution. So it is essential to do a molecular genetic characterization of the natural genetic resources to be utilized in any breeding programme (Chong et al., 2000).

Application of DNA-based approaches to population genetic studies has been limited, probably due to the need for large samples of individuals from each population to provide an accurate estimate of allele and genotype frequencies. The relatively high cost, the requirement for sophisticated equipment and well-trained personnel are other limiting factors in population genetic studies. The RAPD technique has received a great deal of attention from population geneticists (Hedrick, 1992) because of its simplicity and rapidity in revealing DNA-level genetic variation. A major drawback of RAPD markers in population genetic studies is that they are dominant. Thus gene frequency estimates for
such loci are necessarily less accurate than those obtained with codominant markers such as allozymes and RFLPs. Lynch and Milligan (1994) suggested that 2 to 10 times more individuals need to be sampled for dominant markers to achieve the same degree of statistical power as co-dominant markers such as allozymes and RFLPs. The assumption of homology between bands of apparently the same molecular weight from the same primer is potentially another problem for RAPD surveys. Homology between co-migrating bands in different individuals is a good assumption when individuals are from the same population. This may not be true when individuals belong to different species or widely divergent populations (Allegrucci et al., 1995). Because the chance of co-migrating bands being homologous becomes less as populations diverge, it was suggested by Williams et al. (1993) that RAPD analysis gives more accurate estimates between closely related populations and less accurate estimates for distantly related populations.

Research on fish populations has significantly contributed in the development of the science of population genetics, models, and analytical softwares. The effectiveness of RAPD in detecting polymorphism between and among different fish populations, their applicability in population studies, and the establishment of genetic relationships among fish populations has been well documented. Bielawski and Pumo (1997) detected sufficient levels of variation to allow a population genetic analysis of the four migratory populations of striped bass (Morone saxatilis) along the Atlantic coast. Chong et al. (2000) represented the first application of the amplified fragment length polymorphism (AFLP) technique and the RAPD technique in the study of genetic variation within and among five geographical populations of the Malaysian river catfish (Mystus nemurus). Nine RAPD primers detected a total of 42 polymorphic markers, respectively. The results of both AFLP and RAPD analysis provided similar conclusions (Chong et al., 2000). PCR-based multi-locus DNA fingerprints also represent one of the most informative and cost-effective measures of genetic diversity and are useful population-level biomarkers of toxicological and other anthropogenic impacts. Bagley et al. (2001) assessed polymorphism and reproducibility of two common fingerprinting techniques, RAPD and AFLP, in pedigreed populations of rainbow trout (Oncorhynchus mykiss) to derive general rules for selective removal of problematic fingerprint bands. They found that by
excluding bands that comprised less than 1% of total intensity, and by excluding the largest and smallest 10% of the bands, they could achieve nearly 100% reproducibility of AFLP fingerprints. Similar application of band exclusion criteria to RAPD fingerprints did not significantly enhance their reproducibility, and at least 15% of RAPD bands were not fully repeatable, heritable, or transmittable. The RAPD technique produced more polymorphic fingerprints than AFLP. Yoon and Kim (2001) studied genetic similarity and diversity within and between populations from two geographical areas by RAPD-PCR in cultured catfish (*Silurus asotus*).

Dergam *et al.* (2002) conducted a study using RAPD-PCR analysis in order to test for genetic differentiation in populations in Rio Doce basin of southeastern Brazil and for biogeography relationships among populations of *Hoplias malabaricus* (Pisces, Teleostei) in other basins. In the Rio Doce basin, the patterns of genetic similarity of RAPD-PCR markers (individual fingerprinting and Nei’s genetic distance) suggest the existence of two genetically different groups, one composed of the lacustrine populations Carioca and Dom Helvecio, and the other of riverine and the remaining lacustrine populations. Leuzzi *et al.* (2004) used RAPD technique to analyze the genetic structure of populations of the fish *Astyanax altiparanae* (Characidae, Tetragonopterinae) living in the lower, middle and upper Paranapanema river, Brazil. Ten RAPD primers were used in a comparative analysis of the four sites, resulting in 124 bands (loci) in all. The aim was to assess this structure regarding fish handling and conservation programmes. All of the genetic parameters used, indicate that the population in the lower Paranapanema is genetically different from those in the middle and upper sections. The data obtained in this work suggested that recolonization and conservation studies should not be focused on the species *A. altiparanae* as such, but on the conservation units, because they are the genetically differentiated populations.

Nagarajan *et al.* (2006) used 6 RAPD primers to detect genetic differences in *Channa punctatus* populations and a total of 42 loci were amplified out of which 14 were polymorphic bands and 6 were population specific band (unique band). Zelenina (2006) did comparative study of the population structure and population assignment of sockeye salmon *Oncorhynchus nerka* from West Kamchatka based on RAPD-PCR and
microsatellite polymorphism. Using two types of molecular markers, a comparative analysis of the population structure of sockeye salmon from west Kamchatka as well as population assignment of each individual fish were carried out. The values of a RAPD-PCR based population assignment test (94–100%) were somewhat higher than those based on microsatellite data (74–84%). However, these results seem quite satisfactory because of high polymorphism of the microsatellite loci examined. The UPGMA dendrograms of genetic similarity of three largest spawning populations, constructed using each of the methods, were highly reliable, which was demonstrated by high bootstrap indices (100% in the case of RAPD-PCR; 84 and 100%, in the case of microsatellite analysis), though the resultant trees differed from one another. The different topology of the trees is explained by the fact that the employed methods explored different parts of the genome; hence, the obtained results, albeit valid, may not correlate. Muneer et al. (2008) confirmed the suitability of RAPD markers for the study of population genetic structure in yellow catfish Horabagrus brachysoma stocks. Phale et al. (2009) found RAPD assay to be useful for establishing genetic relationship, genome specificity and phylogeny among wild species of Indian major carps.

2.2 Morphometric Characterization Studies
Understanding the origins, maintenance and consequences of variation is a fundamental part of biological research, and requires that variation be both precisely and accurately estimated. Complex variation associated with body form is one of the most difficult types of variation to quantify, and the methods used to assess it are collectively referred to as morphometrics. These methods are concerned with quantifying shape variation within and among samples usually to address developmental and evolutionary questions relating to shape change during growth. The most common approaches, referred to as ‘traditional’ morphometrics, are only a few decades old (Marcus, 1990; Rohlf and Marcus, 1993; Adams et al., 2004), and typically apply multivariate statistical methods (e.g., principal components analysis, canonical variate analysis, discriminant function analysis, or multivariate analysis of variance) to a set of variables measured on each individual. Frequently, these variables are linear distances, often called ‘trusses’ (Strauss and Bookstein, 1982), measured between pairs of landmarks on the body, or body parts, of
each individual (angles among sets of three landmarks are also sometimes used; Meyer, 1987). Most landmark-based approaches involve identifying points that are homologous across the variant forms included in the study. Increased computing power drove the evolution of traditional morphometrics in the 1960s and 1970s that permitted the simultaneous analysis of multiple traits, and so was an obvious improvement over univariate approaches (e.g., Jolicoeur, 1963). However, limitations relating to these traditional methods became increasingly obvious and spurred a ‘revolution’ in morphometrics during the 1980s and 1990s characterized by a shift in focus to the relative geometric positions of landmarks (Rohlf and Marcus, 1993; Bookstein, 1996; Adams et al., 2004). Five basic limitations drove the most recent morphometric revolution. First, linear truss lengths are generally strongly positively related to body size. Different methods of removing the effect of size variation from shape variation are possible but these can lead to different estimates of subsequent ‘shape’ variation and no standard size adjustment method was ever developed (e.g., Reist, 1985; Marcus, 1990). The size problem is inherent to any data derived from landmarks including the newer geometric approaches, because the geometric coordinates of landmarks are influenced by body size. Second, variation among samples becomes more difficult to assess when homologous landmarks do not define trusses. For example, the trait ‘maximum body depth’ may not be indifferent to allometric differences among populations (ANCOVA using size as a covariate is a better alternative). Traditional multivariate morphometrics, accounting for variation in size and shape, have successfully discriminated many fish stocks. Cadrin and Friedland (1999) highlighted the utility of image processing techniques for morphometric analysis and stock identification.

A number of studies have dealt with the morphological variation of teleost populations. Many of these studies are based on the analysis of morphometric and meristic characters. Such characters are useful in defining external morphology and show that population-specific morphological traits often differentiate populations from distinct drainage or marine regions (Wilde and Echelle, 1997; Jerry and Cairns, 1998; Mamuris et al., 1998; Riffel and Schreiber, 1998; Saborido-Rey and Nedreaas, 2000; Brinsmead and Fox, 2002; Cakic et al., 2002; Neves and Monteiro, 2003; O’Reilly and Horn, 2004).
Cavalcanti et al. (1999) used landmark based morphometric analysis in selected species of serranid fishes (Perciformes: Teleostei). Morphological differences among 6 species of marine fishes belonging to 2 subfamilies of the family Serranidae were studied by the geometric morphometric method. Significant differences were found among species with respect to the uniform components, but there is no clear separation of taxonomic groups related to these components, and species are instead separated on the basis of body height and caudal peduncle length. Non-uniform changes in body shape, in turn, clearly differentiate the species of Serranidae and Epinephelinae. These shape changes are probably related to differences in habitat and feeding habits among the species. Morphometric data discriminated the genetically divergent Atlantic and Mediterranean populations of Aphanius iberus (Valenciennes) (Doadrio et al., 2002). More recently, a geometric morphometric analysis showed notable phenotypic divergence among four Pacific coast populations of silverside Atherinops affinis (Ayres), a species that appears to have low intrapopulation genetic variation and that may be phenotypically plastic in response to differences in the environmental conditions where the development of fish occurs (O’Reilly and Horn, 2004). Strauss (1985) elucidated evolutionary allometry and variation in body form in the South American catfish genus Corydoras (Callichthyidae). Studies by Rohlf and Marcus (1993) outlined that morphometrics essentially deal with methodology for the statistical study of shape variation and the co-variation of shape with intrinsic (morphogenetic) and extrinsic (ecological and evolutionary) causes.

Hard et al. (2000) provided evidence for morphometric differentiation of wild and captively reared adult coho salmon using geometric analysis. As part of a comprehensive genetic evaluation of reproduction in naturally spawning coho salmon, Oncorhynchus kisutch, they examined morphometric variation in captivity reared and wild adults from Hood Canal, Washington (U.S.A.) for evidence of differentiation between these groups. Multivariate analysis of shape variation indicated that the captivity reared adults were differentiated from the wild fish by sharply reduced sexual dimorphism as well as smaller heads and less hooked snouts, increased trunk depth, larger caudal peduncles, shorter dorsal fins, larger hind bodies and a reduction in body streamlining. The magnitude and
pattern of differences suggested that at least some of them were environmentally induced. Morphometric variation was a poor correlate of most spawning behaviours.

Rueber and Adams (2001) quantified morphological variation in body shape and trophically associated traits among cichlids using linear measurements, meristic counts and landmark-based geometric morphometrics. A canonical variate analysis (CVA) delineated groups consistent with dentition characters. When the phylogenetic relationships among taxa were taken into account using comparative methods, the covariation of body shape and trophic morphology persisted, indicating that phylogenetic relationships were not wholly responsible for the observed pattern. They hypothesize that trophic ecology may be a key factor promoting morphological differentiation and postulate that similar body shape and feeding structures have evolved multiple times in independent lineages, enabling taxa to invade similar adaptive zones. Jawad (2001) compared meristic characters in samples of the tilapian fish, *Tilapia zilli*, collected from lake Ain Ziana, lake Tawrqa, and Ojala area, Libya. The significant difference in the number of vertebrae and the pectoral fin rays provided an evidence of the existence of three general groups or populations in the three study areas.

Trapani (2003) investigated body-form variability in the trophically polymorphic Cuatro Cienegas cichlid, *Cichlasoma minckleyi* using geometric morphometrics and concluded that the best explanation hinges on the relative importance of genetic and environmental factors in influencing body-form.

Szlachciak (2005) investigated morphological features of 40 specimens of the zope (blue bream), *Abramis ballerus*, from the middle stretch of the Odra River, western Poland, to shed a new light on the phylogeny of this group of fishes. The aim of this study was to learn and describe meristic and morphometric features of zope and to compare the results with published data. Turan et al. (2005) examined the pattern of morphometric differentiation among six populations of *Clarias gariepinus* sited in the Asi, Seyhan, Ceyhan, Goksu, Aksu, and Sakarya river systems in Turkey. The proportion of correctly classified individuals into their original group was highest in the Sakarya sample (93%) and high in the Goksu (88 %) and the Ceyhan (86 %) samples, indicating that these
samples are highly divergent from each other. Consequently the present analysis suggested high morphological differentiation among *C. gariepinus* populations. The detected differentiation may be related to differential environmental conditions such as temperature, turbidity, food availability, and water depth. On the other hand the detected high phenotypic differences between the rivers may depict that the divergent populations may belong to other taxa.

Tomecek *et al.* (2005) studied external morphology of native Canadian (river Otonabee, Looncall Lake) and non-native Slovak (river Danube) pumpkinseed using both triple regression analysis (distance-based measurements) and geometrical analysis (coordinate-based measurements) within an ontogenetical aspect. This study suggested that in the pumpkinseed examined, ontogenetic variations in external shape (a function of epigenetic mechanisms) depend on environmental conditions (in this case riverine compared with lacustrine) rather than on geographical and genetical isolation. In other words, the environmental conditions seemed to be responsible for most of variability in morphology. Indeed, in native population of pumpkinseeds as much as 53% of morphological plasticity was found to be caused phenotypically (by environmental conditions) and only 14% genetically.

Suresh *et al.* (2006) studied biology and fishery of barred spiny eel, *Macrognathus p ancalus* (Hamilton). The aim of this study was to provide necessary inputs on food habits, reproductive biology, and fishery of the species for artificial propagation programmes and fisheries guidelines for conservation of its natural populations. Kovac *et al.* (2006) evaluated the external morphometry of spirlin, *Alburnoides bipunctatus*, a threatened species in parts of its native range; and it was re-examined in specimens from the river Rudava, Slovakia, using geometrical shape analysis, and the relationships between morphometry and habitat use. Adults had proportionally smaller eyes, deeper body, and longer preanal part of the body than the early and ‘middle’ juveniles. These differences tended to coincide with the differences observed in microhabitat use of spirlin, namely a generally increasing preference for high velocity areas with increasing age of the fish. Thus, changes in external morphometry occurring during the ontogeny of spirlin might reflect an increasing affinity for more complex, lotic microhabitats as well
as developments associated with sexual maturation.

Ferrito et al. (2007) highlighted morphological variation among populations of *Aphanius fasciatus* (Teleostei, Cyprinodontidae). The amount of osteological variation among 11 Italian killifish *A. fasciatus* populations was examined by the univariate and multivariate analysis of 40 morphometric and meristic variables of the skull and vertebral column. The statistical analysis confirmed that several populations were well differentiated. In particular, discriminant analysis revealed a strong discriminating power of the morphometric variables. Morphometrics of the vertebrae, bony elements of the pharyngeal jaws, supraoccipital and parasphenoid were the most important in discriminating populations.

Bush and Adams (2007) applied a novel statistical approach to Arctic charr using phenotypic variation to determine conservation value. There is a very high degree of discrete variation in phenotype between populations of Arctic charr. This takes the form of variation not only in morphometric and meristic characters traditionally used to distinguish species, but also in characteristics of life-history, behaviour, coloration and ecology. This variability has a number of consequences, one of these is that there is a strong case for the conservation of populations with extreme phenotypes. However, if variation is discrete between populations but continuous across many populations, this poses difficulties in separating those populations of high conservation value from those of lower conservation value. In this paper they described a statistical technique which enables populations on the extreme edges of the range of phenotypic variation to be identified and applied this to the morphometric characters of charr from 25 populations from across Scotland and Ireland. The technique enabled the identification of any proportion of the most extreme phenotypes. This technique can potentially be used on any species and on any suite of characteristics as an objective measure of conservation value of a population within a continuous phenotypic range.

Wagle et al. (2008) examined morphometric diversification between three river populations (Koshi, Trishuli, Kali Gandaki) and one lake population (Phewa lake) of mahseer. It was examined to identify intraspecific unit for enabling better management
and perpetuation of the resources on Mahseer (*Tor putitora*) from a mid-hill lake and rivers of Nepal. They hypothesized that differences between habitats (e.g. flow regime, foraging opportunities) might create selective pressures resulting in morphological divergence between intraspecific populations. Morphometric analysis showed that most of the shape variation among these populations occurs in the head region, body depth and fin length. Lake population of mahseer was found to diverge most from river populations. The characters that best discriminated the river and lake population of mahseer were associated with locomotion patterns and foraging behavior of fish. It was concluded that mahseer may be phenotypically plastic in response to the environmental conditions of the habitat of each population.

### 2.3 Studies including both Morphometric and Molecular Tools

Assessing overall genetic variability through morphological variation alone is a highly indirect method, because of its being greatly influenced by selection and other environmental factors, thus it may not necessarily reflect the patterns detectable at molecular level. Correlations between genetic variation and morphological variation have been confirmed in natural populations (Soule and Zegers, 1996) and both have been used to make similar assessment of population differentiation (Sugg *et al.*, 1997).

Corti and Crosetti (1996) studied geographic variation in the grey mullet (*Mugil cephalus*) using a geometric morphometric analysis in which multivariate examination of partial warps allowed the description of shape differences characterizing each of 10 populations of *M. cephalus*. Patterns of morphometric distances (overall, uniform and non-uniform) among samples suggested that morphometry in part reflected the geographic origin of the samples. However, comparisons with allozymes and mt DNA provided no exhaustive evidence for a pure phylogenetic cause for the observed patterns of geographic variation.

Agnese *et al.* (1997) did morphometric and genetic characterization of sympatric populations of *Clarias gariepinus* and *C. anguillaris* from Senegal to confirm the presence of two species, *C. gariepinus* and *C. anguillaris*. The two species were closely related genetically and no diagnostic loci were found in allozymes and microsatellites.
studies. Two of the 11 haplotypes of mt. DNA observed were shared by both species. Three of the four assays (morphometry, allozymes and microsatellites) allowed a precise characterization of both. One specimen occupied an intermediate position in the analysis of the data; it was considered an F1 hybrid whose possible origin is discussed.

Rognon et al. (1998) investigated morphological characters and electrophoretic polymorphism at 25 protein loci in nine wild populations of the African clariid catfish *Clarias gariepinus* and seven wild populations of *C. anguillaris*. Two other clariid species, *Clarias albopunctatus* and *Heterobranchus longifilis*, were used as outgroups in the allozyme study. Morphometric and allozyme data are congruent for the Nilo-Sudanian populations of *C. gariepinus* and *C. anguillaris*. Both approaches also distinguished two groups amongst the *C. gariepinus* populations, one containing Nilo-Sudanian populations and the other including Lake Victoria and southern African populations. However, allozyme data suggest that *C. gariepinus* is not a monophyletic group and show that *C. albopunctatus* is more divergent from *C. gariepinus* and *C. anguillaris* than it is from *H. longifilis*, stressing the need for a revision of clariid systematics. The variation observed in *C. gariepinus* is discussed in terms of palaeogeographical events and its use in aquaculture. This study exhibited the morphometrical and the allozyme study to nine populations of *C. gariepinus* and seven populations of *C. anguillaris* sampled throughout the distributional ranges of these species in order to quantify their intra and interspecific variation and to retrace the genetic relationships between populations of both species over a large geographical scale.

Poulet et al. (2004) studied genetic and morphometric variations in the pikeperch (*Sander lucioperca* L.) of a fragmented Delta. They used morphometric and meristic features, otolith shape descriptors and protein electrophoresis in order to assess whether Camargue delta, houses one or several pikeperch populations. All characters except the meristic counts highlighted the existence of two subpopulations: one in the drainage network and one in the irrigation network. Electrophoretic investigation showed that the irrigation network population is closer to the Rhone population and that the drainage network population displayed a high inbreeding rate.
Tzeng et al. (2007) analyzed morphometry and mitochondrial DNA sequences from two *Trichiurus* species in waters of the western North Pacific for taxonomic assessment and study of population structure. The species status of cutlass fishes (*Trichiurus* spp.) in waters of the western north Pacific (five hydrographic areas) was examined using the full-length (consisting of 1141 base pairs) DNA sequence of the mitochondrial cytochrome b gene (mt Cyt b) and truss network-based morphometrics. Two historically confusing *Trichiurus* morphs were suggested to be two separate species because a decisive difference in the genetic and morphometric data between *T. japonicus* and *T. lepturus*, using a restricted congener, *T. brevis* as the out-group was found. Both maximum parsimony and neighbour-joining distance trees supported clear branches for *T. japonicus* and *T. lepturus* at 100% bootstrap support. Discriminant function analysis with adjusted body size performed on the morphometric data of *T. japonicus* and *T. lepturus* revealed a decisive specific gap of non-overlapping scattering. Combining mt Cyt b findings with documented geological events in the western north Pacific, the authors postulated that *T. japonicus* was evolved peripherally from *T. lepturus* in middle Miocene, and these two partially sympatric species were achieved secondarily after the Pliocene when intermediate temperatures were located in the subtropical transitions.

Anderson and Mcdonald (2007) did morphological and genetic investigations of two western Gulf of Mexico menhadens (*Brevoortia* spp.). In this study, meristic counts resulted in two of three characteristics that clearly distinguished species, and genetic differences included 1) near fixation for different alleles between species at two of five loci and 2) significantly large divergence between species at four loci. Vetesnik et al. (2007) did morphometric and genetic analysis of *Carassius auratus* from an artificial wetland in Morava river floodplain, Czech Republic. A study by Castilho et al. (2007) of morphological and mitochondrial DNA divergence validates blackmouth, *Galeus melastomus*, and Atlantic sawtail catsharks, *G. atlanticus*, as separate species. A total of 60 morphometric traits and nucleotide sequences of the entire mt. DNA NADH dehydrogenase subunit 2 (ND2) gene (1047 bp) in 23 individuals of blackmouth, *G. melastomus*, and 13 individuals of sawtail catsharks, *G. atlanticus*, caught in southern Portugal, were examined to test the validity of these two taxa.
Adams et al. (2007) described that the patterns of phenotypic and genetic variability show hidden diversity in Scottish Arctic charr. This study examined the degree and pattern of variability in trophic morphology in Arctic char (Salvelinus alpinus L.) at three spatial scales. The pattern of both phenotypic and genotypic variation suggests a mosaic of variation across populations with geographically close populations often as distinct from each other as populations with much greater separation. Very low levels of effective migrants between populations, even within the same catchment, suggest that this variation is being maintained by very low straying rates between phenotypically and genetically distinct populations, even when there is no apparent barrier to movement. The results proved that the population (rather than the species) makes a more rational unit for the consideration of conservation strategies and that the habitat requirements and therefore management needs may differ significantly between populations.