Chapter 1
INTRODUCTION

Contents

1.1 Scope of the study
1.2 Objective of the study
1.3 Technical programme of the study
1.4 Description of the species
The Western Ghats is 1600Km long, unbroken chain of mountains along the west coast of Peninsular India. Geographically the Western Ghats (steps of a staircase) extends from the mouth of the River Tapti (in Gujarat; about 8°N) to the tip of south India (Kanyakumari, Tamil Nadu; about 21°N). It has been rightly recognized as one of the 34 globally identified 'hot spot' areas of mega biodiversity for conservation and one of the three such areas in the country. With respect to freshwater fish species, the streams and rivers originating from the Western Ghats have been identified as one of the few sites in the world exhibiting high degree of endemism and exceptional bio-diversity (Myers et al., 2000). There are around 326 species of primary and secondary freshwater fishes in the Western Ghats of which nearly 69% (228 species) are endemic to the region (Gopalakrishnan & Ponniah, 2000).

The family Cyprinidae is the largest of freshwater fishes and, with the possible exception of Gobiidae, the largest family of vertebrates (Nelson, 1994). The common name for the family most frequently used in North America is minnow, while in Eurasia it is carp. Various members of this family are important as food fish, as aquarium fish, and in biological research (Nelson, 1994). In this study, a fish species from this family exclusively found in the west flowing rivers originating from the Western Ghat region – *Gonoproktopterus curmuca* – was taken for population genetic analysis.

In spite of rich piscine diversity in the Western Ghats region, practically no attention has been paid for the stock assessment, sustainable utilization and conservation of these species. Several endemic food and ornamental fishes of the region have been enlisted as endangered, either due to over exploitation, gratuitous destruction of spawners, dynamiting or construction of dams (Anon, 1998). Attempts to promote aquaculture practices in the area using transplanted Indian major carps and other exotic species have led to further deterioration of the situation. These waters are also considered as the gold mine for nearly 110 endemic ornamental fishes like loaches, bagrid catfishes and cyprinids. But, recent
surveys reported their alarming rate of depletion due to over-exploitation and clandestine export (Ponniah and Gopalakrishnan, 2000). It is noteworthy that steps have been initiated to conserve the endemic food and ornamental teleosts of the region through propagation assisted rehabilitation programme by the National Bureau of Fish Genetic Resources (NBFGGR), Lucknow (Annamercy et al., 2007). *G. curmuca* is one of the prioritized species for the rehabilitation programme.

1.1 Scope of the study

The water bodies in the form of oceans, rivers, lakes etc., have been exploited by man since time immemorial for the augmentation of food production. The heavy and sometimes ruthless exploitation has even caused extinction of many of the aquatic flora and fauna. There was an urgent need for restoration ecology by the development of apt management strategies to exploit resources judiciously. One of the strategies thus developed for the scientific management of these resources was to identify the natural units of the fishery resources under exploitation (Altukov, 1981). These natural units of a species can otherwise be called as ‘stocks’. A stock (Shaklee et al., 1990b) can be defined as “a panmictic population of related individuals within a single species that is genetically distinct from other such populations”.

The study of genetic variation in fishes has proven valuable in aquaculture and fisheries management, for identification of stocks, in selective breeding programmes, restoration ecology and for estimating contributions to stock mixtures. Moreover, an efficient use of biological resources requires a thorough knowledge of the amount and distribution of genetic variability within the species considered. Generally, individuals with greater genetic variability have higher growth rates, developmental stability, viability, fecundity, and resistance to environmental stress and diseases (Carvalho, 1993). It is believed that a species may undergo microevolutionary process and differentiate into genetically distinct sub-populations or stocks in course of time, if reproductively and geographically isolated. In recent times, there has been a widespread
degradation of natural aquatic environment due to anthropogenic activities and this has resulted in the decline and even extinction of some fish species. In such situations, evaluation of the genetic diversity of fish resources assumes importance. A proper knowledge of the genetic make-up and variability of the fish stocks will help us in the management, conservation of endangered species and improvement of stocks of cultivable species. If the population genetic structure of a species is known, the distribution of subpopulation in mixed fisheries can also be estimated easily. The dearth of knowledge about the genetic structure of the populations may result in the differential harvest of the populations that will ultimately have a drastic and long-term effect. To overcome this, there is always a need for investigation encompassing the genetic variations at the intra and inter-population levels as well as at the intra and inter-specific levels of the fish and shellfish resources of any nation (Allendorf and Utter, 1979).

For the accomplishment of above objectives, scientists all over the world developed different methodologies to distinguish and characterize the fish stocks and evaluate the genetic variation. One of the traditional methods of distinguishing fish stocks has been the comparative examination of morphological characters (Hubbs and Lagler, 1947). But the conventional morphometric measurements have been graded as inefficient and biased, as they often produced uneven areal coverage of the body form. Most of the landmarks were repetitive and unidirectional lacking information of depth and breadth of the body forms (Strauss and Bookstein, 1982; Sathianandan, 1999). This had led to the development of a new method called as truss network analysis, where the shape of the body forms of fish or shellfish also was taken into account along with the size (Humphries et al., 1981; Winans, 1984). However, the application of truss network analysis for the identification of stock is as complicated as the morphometric measurement. The reason for this is the role of non-genetic factors in determining the variability of morphological characters.
In the mid fifties, protein electrophoresis (Smithies, 1955) and histochemical staining method (Hunter and Markert, 1957) gained advantage over morphological studies by providing rapidly collected genetic data. This method is capable of unveiling the invisible differences at the molecular level as visible biochemical phenotypes through allozyme electrophoresis. Allozymes are the direct gene products, coded by a single locus, and often appear in different molecular forms. Any detectable change at the allozyme level reflects the genetic change in the nucleotide sequence of DNA. This genetic change is heritable in Mendelian fashion and the pattern of allozyme gene expression is co-dominant type (Ayala, 1975). The results of a limited number of studies using allozyme electrophoresis demonstrated that 15-30% of structural gene loci were detectably variable within populations, and that even closely related species showed extensive genetic divergence (Hubby and Lewontin, 1966; Harris, 1966). These characteristics make allozymes superior markers over morphological characteristics. Stock identification of several species has been carried out using the above mentioned techniques (Ferguson, 1980; Shaklee et al. 1990; Ferguson et al., 1995; O'Connell and Wright, 1997; Rossi et al., 1998). Allozymes were also found to be helpful in generating species-specific profiles and resolving taxonomic ambiguities in several species (Rognon et al., 1998; Gopalakrishnan et al., 1997; Menezes, 1993; Low et al., 1992; Menezes et al., 1992; Menezes and Taniguchi, 1988; Pouyaud et al., 2000).

The amino acid substitutions of protein detected by electrophoresis are indirect reflections of the actual base substitutions in base sequences. Furthermore, all base substitutions do not necessarily result in change of amino acids and all amino acid substitutions do not result in protein change that are electrophoretically detectable. It has been estimated that only about one third of the amino acid substitutions are detected under the conditions used to collect electrophoretic data in most laboratories (Lewontin, 1974). It is apparent from the above facts that the electrophoretic identity of proteins does not necessarily mean identity of base sequences in DNA. The vast majority of DNA within the nucleus does not code
for protein products and therefore, probably do not affect the fitness of an individual fish. Thus, these non-coding DNA sequences are under relaxed selective constraints and may be free to evolve much more rapidly than the coding sequences.

With the advent of thermocyclers the amplification of small fragment of DNA through Polymerase Chain Reaction (PCR) gained popularity. This enabled the users to screen the polymorphism in the DNA of the individuals without sacrificing them. One such technique (Williams et al., 1990 and Welsh and McClelland, 1990) was Random Amplified Polymorphic DNA (RAPD) based on PCR using short single primers of arbitrary nucleotide sequence typically of length of ten (deca(pri)mers) nucleotides that amplified random segments of the genome. The amplified fragments are also inherited in Mendelian fashion, like allozyme markers (Williams et al., 1993; Bardakci and Skibinski, 1994; Appleyard and Mather, 2000). RAPD fingerprinting has been used recently in many studies for the analysis of phylogenetic and genetic relationship among organisms (Stiles et al., 1993; Bardakci and Skibinski, 1994; Orozco-castillo et al., 1994; Van Rossum et al., 1995). Amplified fragment length polymorphism (AFLP) is another advanced technique suitable for finger-printing simple and complex genomes from different species (Vos et al., 1995; Felip et al., 2000). In AFLP, genomic DNA is digested by restriction endonucleases and amplified by PCR using primers that contain common sequences of the adapters and one to three arbitrary nucleotides as selective sequences (Lin and Kuo et al., 1995).

Variable Number of Tandem Repeats (VNTRs) include minisatellites and microsatellites. Minisatellites are DNA sequences usually 10-200 bp long that are repeated in tandem at variable number of times. Microsatellites are the tandemly repeated DNA sequences with repeat size of 1-6 bp repeated several times flanked by regions of non-repetitive DNA (Tautz, 1989). These are highly polymorphic in nature and be analyzed with the help of Polymerase Chain Reaction (PCR). They are another type of powerful DNA marker used for quantifying genetic variations
within and between populations of species (O'Connell et al., 1997) and also at individual level especially in forensics and paternity disputes.

The mitochondrial DNA (mtDNA) is another type molecular marker, which revealed high levels of sequence diversity at the species and lower levels, despite great conservation of gene function and arrangement (Avise and Lansman, 1983; Brown, 1985). Mitochondrial DNA is smaller, double-stranded and is typically made up of only 16000-20000 nucleotides (Brown, 1983). Initial surveys to detect informative polymorphisms may involve the use of a large number (10-30) of restriction enzymes, but once diagnostic polymorphisms have been identified, only those informative enzymes need be used in subsequent screening. As it is maternally inherited, the analysis of maternal lineage can be done with ease. The use of mtDNA proteins and more recently PCR amplifications of selected regions followed by sequencing the PCR products have made the examination of mtDNA variations considerably easier and faster. The slow-evolving regions of mtDNA such as 16SrRNA are used to discriminate species and higher levels of taxa while fast evolving zones such as control region (D-loop) and ATPase genes are used in population genetic analysis. Universal vertebrate primers can be used to amplify various mtDNA regions and with the advent of recent mtDNA sequences for several fish species being available, more fish specific primers can be designed.

In brief, the techniques available to screen the variability at different levels of the species organization are many ranging from simple morphometric to molecular genetic methods that can reveal polymorphism at the DNA level. The species that is selected in the present investigation for applying three molecular genetic markers (allozymes, RAPD and microsatellites) is the red-tailed barb, *Gonoprotcterus curmuca* from three rivers (viz., Periyar River, Chalakkudy River, and Chaliyar River) originating from the Western Ghats. The major reasons for selecting this particular species are given below.

*Gonoprotcterus curmuca* (Figure 01 & 02) belongs to Family Cyprinidae and is endemic to the rivers originating from southern part of the biodiversity hotspot –
the Western Ghats. The species enjoys a good market value as a food fish and fetches Rs.70-100/Kg in Kerala. Owing to its fast growth rate (maximum size 70cm total length), it is one of the potential candidate species for aquaculture practices in the region. Its attractive colour makes it an ideal species for aquarium keeping in India and abroad (fetches US $ 10 per live fish in international market). Till date, stock assessment of the species has not been made in different rivers; hence there is no information about the current exploitable potential of red-tailed barb. However, there has been a massive hunt for the species from wild for aquarium trade since last few years and its drastic decline was recorded in 1997 itself in field surveys. The workshop on Conservation Assessment Management Plan (CAMP) to evaluate the status of freshwater species of India, held in 1997 categorized this species as "endangered" based on latest IUCN criteria due to restricted distribution, loss of habitat, over exploitation, destructive fishing practices and trade (Anon., 1998). The species was finally short-listed as one of the candidates for stock-specific, propagation assisted rehabilitation and management programme in rivers where it is naturally distributed. In connection with this, captive breeding and milt cryopreservation techniques of the species have been developed by the National Bureau of Fish Genetic Resources (NBFGR), Lucknow. However, for a scientific stock-specific rehabilitation programme, information on the stock structure and basic genetic profile of the species are essential and that is not available in case of G. curmuca. In view of the above facts and reasons, the present work was taken up (1) to identify molecular genetic markers like allozymes, microsatellites and RAPDs in G. curmuca and, (2) to use these markers to discriminate the distinct populations of the species, if any, in areas of its natural distribution.

1.2 Objective of the study

Population genetic analysis of natural populations of Gonoproktoperus curmuca from its distributional range using allozymes, microsatellites and RAPDs.
1.3 Technical programme of the study

a. Identification of allozyme and RAPD markers to be used for stock discrimination of *Gonoproktopterus curmuca*.

b. Identification of microsatellite marker by cross-species amplification of primer sequences of other closely related fish species (derived from available accessions in GenBank or from available literature) for using them as potential genetic markers in *G. curmuca*.

c. The population structure analysis of *G. curmuca* using allozymes, microsatellites and RAPDs.

1.4 Description of the species

1.4.1 Taxonomic status

*G. curmuca* (Figure 01 & 02) is a freshwater barb described by Hamilton-Buchanan in 1807. The species has following synonyms: *Barbus curmuca*, *Hypselobarbus curmuca*, *Puntius curmuca*. The current taxonomic position of *G. curmuca* according to Talwar and Jhingran (1991) and Jayaram (1999) is given below.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Vertebrata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subphylum</td>
<td>Craniata</td>
</tr>
<tr>
<td>Superclass</td>
<td>Gnathostomata</td>
</tr>
<tr>
<td>Series</td>
<td>Pisces</td>
</tr>
<tr>
<td>Class</td>
<td>Teleostei</td>
</tr>
<tr>
<td>Subclass</td>
<td>Actinopterygii</td>
</tr>
<tr>
<td>Superorder</td>
<td>Acanthopterygii</td>
</tr>
<tr>
<td>Order</td>
<td>Cypriniformes</td>
</tr>
<tr>
<td>Family</td>
<td>Cyprinidae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Gonoproktopterus</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>curmuca</em></td>
</tr>
</tbody>
</table>
1.4.2 Confusion over the scientific name of the species

Hamilton-Buchanan (1807) described *Barbus curmuca* from Vedawati River of the Tungabhadra drainage in Mysore, with two barbels, 39 scale rows along the lateral line and a weak and articulated last undivided dorsal ray. Sykes (1840) described *B. kolus* also with the same characteristics from Deccan. Specimens from South Canara with four barbels and the caudal tipped with black, Day (1878) considered as a local variety of *B. curmuca*. But subsequent workers like Hora and Law (1941), Silas (1951) Talwar & Jhingran (1991), Jayaram (1997, 1999) treated the species with four barbels and weak last undivided dorsal ray and 41-43 lateral line scales as *Puntius curmuca* (later renamed as *Gonoproktopterus curmuca*) and the other species with 2 barbels and 39 scale rows along the lateral line and slate-colouration as *G. kolus*. This classification was widely followed in the standard taxonomic books (Jayaram, 1999; Talwar & Jhingran, 1991; Shaji et al., 2000). Menon & Rema Devi (1995) later renamed the red-tailed barb from Kerala and South Canara as *Hyselobarbus kurali* which was earlier referred to as *G. curmuca*.

To avoid confusion, in the present study the species name of red-tailed barb (4 barbels, 41-43 scales in the lateral line and caudal tipped black) is retained as *Gonoproktopterus curmuca* (Hamilton – Buchanan, 1807) following the standard fish taxonomy books (Jayaram, 1999; Talwar & Jhingran, 1991; Shaji et al., 2000) and the species is having following diagnostic characters.

1.4.3 Distinguishing Characters

\[ D_{iv} 9; A_{iii} 5; P_{i} 15; V_{i} 8. \]

Body fairly deep, the dorsal profile convex and the ventral profile nearly horizontal, its depth about four times in standard length. Snout conical; a band of pores on cheeks. Eyes moderate, its diameter about 4.3 times in head. Mouth sub-terminal; barbels two maxillary pairs, lower ones as long as orbit, upper ones half as long. Dorsal fin inserted anterior to origin of pelvic fins, its last un-branched
ray osseous but weak. Scales medium; lateral line with 41 to 43 scales; lateral transverse 3 ½ to 4 ½; pre-dorsal scales 9.

1.4.4 Colour

In life, silvery, lightest on flanks and belly. Caudal fin with blackish tip; in young middle-third of caudal fin orange, tipped with black.

1.4.5 Common names

The species is commonly known as “red-tailed barb” in English and locally called as "Kooral" or "Chundan" in Malayalam.

1.4.6 Habitat and distribution

*Gonoproktoperus curmuca* is confined to selected west flowing rivers originating from the Western Ghats in the states of Kerala and Karnataka (South Canara). The species once found in abundance has recorded a sharp decline in the catches due to over-exploitation for ornamental fish trade and for human consumption and is now restricted to a few rivers viz., Nethravathi River, Chaliyar River, Bharathapuzha River, Chalakkudy River, Periyar River, Kallada River, Achankovil River. It is usually recorded from the upper middle stretches of these rivers.