Chapter 5

DISCUSSION

Accurate species identification forms the basis of many aspects of biological research (Carew et al., 2005). The first and foremost thing in any detailed scientific study is the exact identification of the organism. Species misidentification may lead to inaccurate estimates of stock size. For the scientific management and conservation of a species it is important to identify geographical distribution and basic genetic characteristics of the isolated populations. Accurate species identification is required for stock assessment to monitor exploited species, as mislabeling may lead to serious errors in stock estimates.

Considerable research has been undertaken in *C. gariepinus* and *C. magur*, but the genetic work conducted on *C. dussumieri* is scanty. The population of *C. dussumieri* has shown evidence of overexploitation and signs of capture decline after 1980’s possibly because of the spread of epizootic ulcerative syndrome, a major disease which affected many groups of fishes in the period and many other reasons. In this background, it is relevant to assess the genetic variability in the wild populations of *C. dussumieri* through molecular markers for the sustainable utilization of this economically important species.

The present study provides a comprehensive genetic analysis of three species of *Clarias* seen in India i.e. *C. gariepinus*, *C. magur* and *C. dussumieri* and genetic stock structure of *C. dussumieri* in three river basins. The present study is the first attempt in determining the molecular phylogeny of Indian clariids, besides the population genetic structure analysis of *C. dussumieri* which is an endemic fish to Southern regions of Western Ghats.
5.1 Molecular Phylogeny of Indian Clariids

5.1.1 Species validity of *C. dussumieri*, *C. magur* and *C. gariepinus*

Molecular genetic information, including mtDNA sequences are very useful for clarifying species boundaries (Avise 1994, 2000). Molecular genetic analysis carried out in the present study using DNA sequences of three mitochondrial genes *viz.*, Cytochrome Oxidase subunit I, Cytochrome *b* and 16S rRNA, had shown unambiguous genetic divergence between these three species.

The mean pairwise genetic divergence between the individuals of three species was zero for all the three genes in all the three species except for *C. magur* from Andaman Islands. All the species tested were represented by a single haplotype for the three tested genes. Only *C. magur* collected from Andaman showed two haplotypes for the COI gene which showed a 0.06% genetic distance between the haplotypes. Out of these two haplotypes, one was shared between India and Andaman.

The mean pair wise distance between the three tested species based on COI gene ranged from 12.8% to 16.5%. Based on the 16S rRNA sequences the distance between the species ranged from 1.8% to 2.58%. The mean pair wise genetic distance in the tested species for the Cytochrome *b* gene ranged from 10.9 to 15.5. Based on the combined dataset of 1780 bp region of the COI, Cyt *b* and 16S rRNA regions, the mean pair wise distance was between 8.3% to 15.6%.

Khedkar et al., (2014 a) obtained sufficiently high average values (K2P) for intraspecific divergence (0.53%) and intra generic divergence (15.05%) to allow differentiation of morphologically challenging specimens of *C. batrachus* and *C. gariepinus* based on the COI sequences. Mwita & Nkwengulila, (2008) obtained uncorrected sequence divergences ranging
from 0.5 to 11.5% between the clariid catfishes from the Lake Victoria, Tanzania.

The pair wise genetic divergence obtained in the present study is in conjunction with many published results. John, (2009) obtained similar results for *Puntius* species from the rivers of Kerala, 4.98% with 16S rRNA, 9.03% with COI, 11.16% with ATPase 6/8 and 14.68% with Cyt *b* sequences. Wong et al., (2011) reported the K2P distance between species ranging from a low 0.8% (Ictalurid hybrids and *I. punctatus*) to a maximum value of 22.6% (*C. macrocephalus* and Ictalurid hybrid) based on COI gene. Bose (2007) reported pair wise divergence ranging 6.28% to 8.87% for 16S rRNA, 13.48% to 16.0% for COI and 15.95% to 18.77% for Cyt *b* sequences respectively among three cyprinid species of the genus *Garra* from the Western Ghats; Based on 16S rRNA sequences, Salini, (2007) obtained a mean genetic divergence value ranging 3.43% to 14.66% among selected cyprinid species from the Western Ghats; Vineeth, (2006) observed pair wise divergence ranging 4.79% to 6.84%, and 16.30% to 21.60% based on 16S rRNA, and COI sequences respectively among three Indian cichlid species and Garcia et al., (2000) reported 4.5 to 28% sequence divergence based on 324 bp of mitochondrial Cyt *b* region in annual killifishes of the genus *Cynolebias*.

*C. magur* collected from Indian mainland and Andaman Islands were included in the study. The analysis of mitochondrial sequence divergence showed that there is no significant genetic difference between the samples of *C. magur* collected from these two geographically isolated regions based on the COI, 16S, Cyt *b* and the combined data set of these three gene sequences. The mean pair wise genetic difference between the haplotypes of two populations ranged from 0.12% for COI, 0.17% for Cyt *b* sequence and 0.09% for combined sequence. There was no pair wise genetic difference
between these two based on the noncoding 16S rRNA sequences. In a study conducted on *C. batrachus* commonly known as magur by Khedkar et al., (2014b), it was found that *C. batrachus* population was lacking genetic diversity in India.

The present study revealed clear difference of within and between species sequence variation based on the multiple mitochondrial sequences. This result is suggestive of the unambiguous differentiation of the morphologically similar species based on mitochondrial DNA sequences.

Three mitochondrial genes were selected for the present study i.e. COI, 16S and Cyt *b*. The protein coding genes COI and Cyt *b* showed significant genetic divergence than 16S rRNA sequence as expected with higher rate of evolution exhibited by those genes. The Cyt *b* region appeared to be the most variable marker with 27.68% divergence trailed by COI in the second position with 17.06% divergence. 16S rRNA gene showed the least divergence i.e. 3.65%. This result was in concordance with the study by John et al., (2009) in *Puntius* sp. The polymorphic sites were having the percentage of Cyt *b* 18.12%, ATPase 6/8 14.61% and COI 13.05%. No indels were noticed in the protein coding genes where as two indels were seen in 16S sequences. Rarity of indels (Mardulyn and Whitfield, 1999) was expected in this region and this was one of the reasons for the use of COI as a gene for ‘barcoding’ animals including teleost species (Hebert *et al*., 2003a).

**5.1.2. Phylogenetic relationships of Clarias species**

Phylogenies from molecular data are often computed by pair-wise genetic distance based (numerical) methods like Neighbor Joining (NJ) tree, with branch lengths that are proportional to the amount of divergence. NJ tree making method is a widely used distance–clustering algorithm that
allows unequal rates of divergence among lineages. Phylogenetic trees are also made based on “discrete methods” that operate directly on sequences like the Maximum Parsimony (MP) tree. MP chooses the tree (or trees) that require the fewest evolutionary changes (i.e. it makes trees from sequences exhibiting smallest evolutionary changes). Invariant characters (bases) those that have the same state in all taxon are obviously unimportant (phylogenetically uninformative) and are ignored by the MP method. Both the numerical (NJ) and discrete (MP) tree making methods are used in the analysis in most of the species (Hall, 2004) as in the present study. Both analyses were carried out separately for the nucleotide data generated from each mitochondrial DNA genes such as COI, 16S rRNA and Cyt b. A combined analysis was also carried out by combining nucleotide data of all the genes together. MP and NJ trees were generated for independent genes and also for the combined gene dataset. All the trees shared a similar topology with significant bootstrap values ranging from 96 to 100. Two major clades were obtained consistently, one clade formed by *C. magur* from Indian mainland and Andaman Islands; the second clade formed by two subclades of *C. dussumieri* and *C. gariepinus*. *C. dussumieri* always appeared associated with the African Catfish *C. gariepinus*.

The results revealed: 1) the phylogenetic trees established the validity of *Clarias* species in India. 2) All the trees uniformly showed a relationship between the African species *C. gariepinus* and Indian species *C. dussumieri*. This result gave a hint towards the well known Afro Asian relationship between some fish species. 3) Estimated the uniform population structure of *C. magur* from India and Andaman. 4) clarified the relationship of *C. magur*, *C. dussumieri* and *C. gariepinus*.

A global phylogeny was made using the COI sequences available in NCBI with our sequences. This study confirmed the relationship of *C.*
**dussumieri** with the African species. In both the MP and NJ trees formed, **C. dussumieri** appeared together with the clade formed by African species. All the African species appeared in a clade and all the South East Asian species appeared in another clade. All individuals of the three species were separated as three major distinct groups exhibiting the phylogenetic separation between the tested **Clarias** species. In the first clade a distinct pattern could be visualized i.e. a group formed by two subclades of **C. dussumieri** and **C. gariepinus**; all the mtDNA genes analyzed reflected a clear association between **C. dussumieri** and **C. gariepinus**. The second clade was formed by **C. magur**. The tested samples included **C. magur** from both Indian mainland and Andaman Island. Both of these samples appeared together in a single clade indicating no population structuring between **C. magur** of Indian and Andaman waters.

### 5.1.3. Intercontinental diversification of catfishes/ African-Asian relationships within catfishes

The topology of ML and NJ trees was congruent. Transitions were more than transversions as expected. Generally for teleost mtDNA, a much larger excess of transitions related to transversion is typically observed (Ward et al., 2005.)

Based on the ML and NJ tree, two major clades were consistently observed with African species in one clade along with **C. dussumieri** and Asian species in the other clade. The two large continental clades, “Asia,” “Africa” suggest a prevalence of intra continental diversification of catfishes. This result is in conjunction with the study of Agnese and Teugels, (2005), who suggested that Clariidae species might have been separated into two groups at an early stage of their evolution, one group containing the African species and the other, the Asian species. Thus the COI phylogeny identifies
the well-known African-Asian relationships within catfishes (Sullivan et al., 2006).

In the Asian species clade four subclades were seen consisting of *C. magur*, *C. fuscus*, *C. batrachus* and *C. macrocephalus*. According to Ng and Kottelat, (2008) the name *C. magur* should be used for the species of *Clarias* occurring in north-eastern India previously identified as *C. batrachus*. This argument is proved in this study as *C. magur* collected from Indian mainland and Andaman Island forms one subclade and *C. batrachus* of South East Asian origin forms another subclade in the Asian clade with 100 bootstrap values.

The African clade differentiates into two subclades with *C. dussenieri* in one subclade and *C. gariepinus* in the other with other African species such as *C. camerunensis* as near immediate sister taxa and *C. jaensis*, *C. angolensis* and *C. gabonensis* as other members.

*C. dussenieri* is showing strong relation with *C. gariepinus*. The simplest explanation for this phenomenon is to consider that the ancestor species of *C. dussenieri* and *C. gariepinus* originated in ancient super continent Gondwana and both of them got separated during continental drift and differentiated into two different species. The mean genetic difference between *C. dussenieri* and other selected African species in this study is 12.8%. According to Agnese and Teugels, (2005) genetic differentiation between Asian and African species was 12.4%. The rate for mitochondrial gene evolution estimated for fishes is 1% sequence divergence per million years (MY) (Dowling et al., 2002; Smith et al., 2002). Based on this theory it can be said that the divergence time between the Asian and African species is 12-13 MY. But, Africa and Asia were disconnected about 160 million years ago (Agnese and Teugels, 2005). Had the two groups separated during
According to Agnese and Teugels, (2005), Clariidae should have originated in Asia because *Heteropneustes* species which represents a sister family of Clariidae, are present only in India.

Pariselle (2003) postulated that African Clariidae should have an Asian origin, based on the study of monogeneans gill parasites of Siluriformes. In Asia, all Siluriformes parasite have a haptor with special sclerotised pieces called “cuneus” where as in Africa the same structure is seen only in parasites from Clariidae species and not in other Siluriformes.

Another proof for the Asian origin of Clariids comes from the paleontological study. Gayet, (1987) reported the oldest known Clariidae fossil of Lower-Middle Eocene (45–50 MY) origin from Pakistan while the oldest African fossils are from Middle Miocene (10–15 MY) (Van Couvering, 1977). Oldest Clariidae fossils are reported from the present Arabian plate also which are of Oligocene period (30 MY) from the Sultanate of Oman (Otero and Gayet, 2001). The same author also assumes that brackish water bridges like lagoons should have preceded the first terrestrial connections between the continents. Some contemporary and wide spread species such as *Clarias anguillaris* and *Clarias gariepinus* are able to live in brackish lagoons as in West Africa (Agnese and Teugels, 2005).

Combining all these principles, it can be postulated that the presently studied species of Clariidae originated in Asia (based on the occurrence of *Heteropneustes* by Agnese and Teugels, 2005 and parasitic study by Pariselle, 2003) 40–50 MY ago (based on fossil records of Van Couvering, 1977). They arrived in the Arabian plate 30 MY ago (fossil record from Saudi Arabia and Sultanate of Oman, Otero and Gayet, 2001). From there
they might haven’t colonized the African part as there are no African fossil record older than 15 MY, but have stayed there until at least the Lower Miocene connection between Asia and Africa, at 18 MY (Otero and Gayet, 2001). Then the ancestral species of studied Clariids might have come back to Asia and colonized Africa at least 10 to 15 MY ago (fossil study).

5.1.4. Evolutionary molecular dating using BEAST software

The divergence date estimates based on fossil data and BEAST analysis suggested that the divergence of the common ancestor of the tested *Clarias* species occurred around 44 MYA in the middle Eocene. The divergence of Asian *Clarias* species occurred in the middle Eocene itself around 35 MYA earlier than the radiation of common ancestor for African *Clarias* around 20.27 MYA. This date confirms the assumption of Agnese and Teugels, (2005) that the ancestor of *Clarias* species originated in Asia and moved to Africa.

5.2. Species differentiation using RAPD

Along with mitochondrial DNA sequence variations, RAPD analysis was also performed for molecular phylogeny in three *Clarias* species in India. In the present study, three *Clarias* species could be successfully differentiated on the basis of species specific markers generated. The technique was found to be useful for establishing genetic relationship and molecular phylogeny.

Average pair-wise genetic distance (GD) based on Nei’s unbiased measures of genetic distances (Nei, 1978) were calculated for eight Operon primers. The values of ‘GD’ between *C. dussumieri* and *C. gariepinus* were 0.7754 and between *C. dussumieri* and *C. magur* was 0.7786. The genetic distance between *C. gariepinus* and *C. magur* was 0.8461. Mean sequence divergence for all pair wise comparisons between three species and the
pairwise GD values based on RAPD analysis in the present study were all significant enough to differentiate these three species as distinct species. This result indicates that RAPD technique is a very useful tool to discriminate the tested *Clarias* species.

The result obtained in the present study based on RAPD is in congruence with molecular phylogeny based on mitochondrial DNA. According to Bhat et al., (2012), estimates of Nei’s unbiased genetic distance in eight Channid species ranged from 0.3292 to 0.800. A genetic distance value of 0.23 to 0.60 is detected in groupers by Roy et al., (2014). A genetic distance value of 0.425 to 0.751 was reported in three mud crab species (Klinbunga et al., 2000). Saini et al., (2011) investigated the phylogenetic relationship of six species of Bagrid catfishes. RAPD was found to be useful for establishing phylogeny among wild species of Indian major Carps also (Phale et al., 2009). Genetic variation of four species of Indian carps was performed by Barman et al., (2003) also.

An unweighted Pair Group Method with arithmetic mean (UPGMA) dendrogram was constructed using the genetic distance values to show the genetic relationships among the three species of *Clarias* using POPGEN Software and the dendrogram showed two clusters, *C. gariepinus* and *C. dussumieri* formed one cluster while the *C. magur* formed another cluster with bootstrap support value of 50.

Morphologically *C. magur* is related to *C. dussumieri* than *C. gariepinus*. *C. gariepinus* is an African native fish and is introduced to Indian waters where as *C. magur* and *C. dussumieri* are South East Asian species. But the genetic analysis using both mitochondrial and RAPD data revealed that genetic relationship was close among *C. dussumieri* and *C. gariepinus*. In conclusion, the RAPD primers have provided consistent
banding pattern and represents a useful and reliable tool for species discrimination and for detecting genetic relationship in *Clarias* species.

The three species of *Clarias* were genetically distinct, as follows. 1) The amount of pairwise sequence divergence within the three species was much less than that between the three species; 2) the degree of sequence difference between the three species was comparable to that existing between many of the teleost species; and 3) in both the MP and NJ trees with all the mtDNA regions analyzed, the haplotypes of the three species were each monophyletic with 96 to 100% bootstrap values 4) Significant genetic difference values were obtained between species based on RAPD analysis.

5.3. Population genetic analysis based on ATPase 6/8 gene

Mitochondrial DNA is usually highly polymorphic than nuclear DNA. Polymorphism should be distinguished from heterogeneity. The former measures the multiplicity of genotypes within a sample whereas in the latter, the probability of two genotypes drawn at random from the sample will be different. Mitochondrial DNA is important in revealing population genetic structuring and is likely to result from the higher evolutionary rate and smaller effective population size. MtDNA heterogeneity is more sensitive to fluctuations in population size ("bottlenecks" and "founder events") than is nuclear heterozygosity (Wilson *et al.*, 1985).

The present study has demonstrated that direct sequencing of variable mtDNA fragments has the potential to provide useful insights into the genetic diversity, divergence and genealogy of *Clarias* populations.

Statistical analysis showed very less difference in molecular variation of *C. dussumieri* populations. The genetic divergence between the Periyar and Chalakudy populations were much lesser than the divergence between these two populations with River Sharavathy. The reason for this
traditionally unexpected result for fresh water populations might be because of the mixing between populations. River Periyar and Chalakudy is closely existing and having connections at the lower stretches. More detailed study has to be done to find out the actual reason behind the reduced genetic diversity within this species. Mitochondrial DNA based population studies has been conducted in many fishes. High degree of population structuring could be established by John et al., 2013 in *Puntius denisonii* where as very less genetic difference was seen in related species *P. chalakkudiensis* based on mitochondrial ATPase 6/8 gene sequence variations. Genetic stock structure analysis of the spiny and slipper lobster populations along Indian coast based on the hyper variable region of COI sequences revealed no significant differentiation in a study done by Jeena, (2013). Relatively low levels of genetic variation was reported by Khedkar et al., (2014 b) in the populations of *C. batrachus* on the basis of Cytochrome *b* and D loop regions of mitochondrial DNA.

5.3.1. Population structure and divergence pattern in *C. dussumieri*

The present study clearly demonstrated that little divergence was observed within and between three riverine populations of *C. dussumieri*. The null hypothesis of panmixia is accepted in the populations of *C. dussumieri* due to less genetic variations between the populations. Both the haplotype diversity (*h*) and the nucleotide diversity (*π*) was generally low ranging in the tested populations. Haplotype diversity ranged from 0.2 (Periyar populations) to 0.4290 in Sharavathy populations where as the nucleotide diversity ranged from 0.00024 (Periyar populations) to 0.00051 (Sharavathy populations). According to Grant and Bowen, (1998), the interpretation for the low haplotype diversity coupled with low nucleotide diversity is recent population bottleneck or founder event by single or a few mitochondrial lineages. The mismatch distributions were performed with the
available haplotypes. The graph obtained were straight lines, but does not fit to an expansion model curve. This result suggested a history of long-term population stasis and a lack of clear expansion leading to localized loss of genetic variation.

\( F \)-statistics and phylogenetic analysis indicate marked uniform structuring in \( C. \) dussumieri populations at the inter-regional scale. Population differentiation depends directly on gene flow. Species with higher capabilities of dispersal and migration across geographic barriers do not show much population differentiation among population over time. In the present study, the \( Nm \) (a maximum of 30.05 between Periyar and Chalakudy populations) values observed were considerably higher indicating that gene flow among spatially distant populations of \( C. \) dussumieri is very frequent. The mean pairwise distances among populations ranged from 0.00027 (between Periyar and Chalakudy) to 0.00045 (between Sharavathy and Chalakudy) also indicated no population structuring within the group.

\( C. \) dussumieri has shown a marked decline in abundance in recent years. Aneesh et al., (2013), categorized \( C. \) dussumieri as vulnerable species based on their sampling information, while the exotic African catfish \( Clarias \) gariepinus (Burchell) was obtained throughout the year. In the present study only 60 specimens could be collected even with frequent sampling. The fish once prevalent mostly in brackish water zones has now become extremely endangered owing to indiscriminate reclamation of the swampy wetlands and with the invasion of the exotic catfish, \( C. \) gariepinus in their natural habitats. The lack of commercial hatchery or commercial captive breeding practice together contributed to the decline of abundance and population. Populations with smaller effective population sizes tend towards rapid fixation of haplotypes, which may be the reason for the reduced number of haplotypes in \( C. \) dussumieri population. The captive breeding technology has been
developed by Padmakumar et al., (2010) and Aneesh et al., (2013) from College of Fisheries, Panangadu, Kerala.

5.3.2. Historical demography of *C. dussumieri* population

Determining the historical demography of a population is an important concern in conservation genetics. While discussing the term population it is important to distinguish between the populations that are small naturally and have limited genetic variation with those which have less genetic variation due to recent reduction in population size due to a past bottleneck (Crandall *et al.*, 1999; Turner *et al.*, 2002). Either the cases it is important to know the past demography of the populations to understand its effect on the current genetic variability which in turn will help for the design of conservation and management strategies of the population. According to Vila *et al.*, (2003), influence of past demography on current genetic variability can have important management implications in terms of the genetic stability of populations and the potential impact of inbreeding depression on population viability. Mismatch distribution of pair wise nucleotide differences together with neutrality tests can be a source of data on recent evolutionary history by inferring past population sizes (Rogers and Harpending, 1992). The distribution of nucleotide site differences between pairs of individuals will give an idea about whether the population was under growth or decline. In histograms showing the distribution of relative frequencies and pair wise mismatch distributions the shape of curves will depend on past demographic events (Rogers and Harpending, 1992).

The relationship between $h$ (haplotype diversity) and $\pi$ (nucleotide diversity) is informative about population demographic history. Taking into account the levels of diversity detected by these two indices, Grant and Bowen (1998) defined four categories of fish, using either mtDNA sequence
data or RFLP: 1) low $h$ and $\pi$ are interpreted as recent population bottleneck or founder event by single or a few mtDNA lineages; 2) high $h$ and low $\pi$ are interpreted as population bottleneck followed by rapid population growth and accumulation of mutations; 3) low $h$ and high $\pi$ are interpreted as divergence between geographically subdivided populations and 4) high $h$ and $\pi$ are interpreted as large stable population with long evolutionary history of secondary contact between differentiated lineages.

For the tested population of *C. dussumieri* both the haplotype diversity and nucleotide diversity was less. According to Grant and Bowen, (1998), low haplotype diversity and low nucleotide diversity indicates a recent bottleneck or founder effect on the past demography of the populations. The observed distribution of pair wise differences between the ATPase 6/8 gene haplotypes was roughly a straight line. But the information from this data was not well fitting to the population expansion model suggesting a history of long-term population stasis and a lack of clear expansion. This pattern of mtDNA variation has been observed in a highly endangered cyprinid species *P. chalakkudiensis* (John, 2009).

Low haplotype and low nucleotide diversity suggested a recent bottleneck or founder effect. The mismatch distributions were not fit to an expansion model curve suggesting a history of long term population stasis. Genetic variability of these populations might have had been influenced by the habitat reduction, exploitation or otherwise not recovered from the bottleneck. The raggedness index of the population indicates the stability of the population.

MtDNA based stock recognition may also have some limitations. The two populations, River Periyar and River Chalakudy are closely existing and having connections at the lower stretches. In order to get a realistic picture of
the population structure of *C. dussumieri* a combined approach using comparatively faster evolving mtDNA control region and highly variable nuclear DNA regions such as microsatellites with adequate sample size will be more appropriate.

The climate and sea-level changes of the last 18,000 years (last glacial maximum, ‘LGM’) wrought great changes on the landscapes of the world in general. The most recent glaciation and the subsequent warming and sea-level rise (17,000–6500 years ago; Neal and Stock, 1986) had significant effects on the intra-specific genetic structuring of many terrestrial and aquatic fauna throughout the world (Hewitt, 2000). Based on this study and several other studies carried out in several fish species of Western Ghats (Muneer, 2005; Johnson *et al*., 2007, Musammilu, 2008) in which significant intra-specific genetic structuring were reported; it may be suggested that the LGM or glaciations prior to the LGM may have had strong impacts on the diversity of aquatic fauna of this region.

### 5.4. Population structure using RAPD

RAPD technique developed by Welsh and McClelland, 1990 is a nuclear type II marker which is a quick and effective method for the detection of genetic polymorphism. In order to determine the genetic diversity among populations of *C. dussumieri* RAPD profiles were generated from three river systems. In order to ensure reproducibility of results which is often cited as a major problem with use of RAPD markers (Liu and Cordes, 2004), high quality DNA and constant experimental conditions and scoring of only bands with frequencies of at least 10% to avoid non-specific amplifications (Castro and Madi-Ravazzi, 2000; Castiglioni and de Campos Bicudo, 2005) were followed.
5.4.1. Genetic variability in RAPD analysis

In this study, RAPD profiles were generated from 30 specimens of *C. dussumieri* collected from various locations of three river basins using 40 arbitrary primers from Operon OPK and OPU series. From these forty primers, eight Operon random primers *viz.* OPK-1, OPK-6, OPK-14, OPK-15, OPU-2, OPU-5, OPU-8 and OPU-18 showed reproducible results with good banding pattern. According to Liu and Cordes (2004), six or seven primers are sufficient to access the genetic variability within and among populations. In the population study of *C. batrachus*, Khedkar et al., (2010) used six primers out of 22 primers for the final analysis. Five out of ten random primers which produced constant and reproducible pattern were selected by Garg et al., (2010) for the assessment of genetic diversity of *C. batrachus* in three water bodies of Bhopal. The number of primers and the sample size used in this study were expected to be adequate to resolve the genetic polymorphism among the *Clarias* populations.

Species specific bands were obtained for *C. dussumieri*, *C. magur* and *C. gariepinus*. All the species specific bands obtained for *C. dussumieri* in molecular phylogenetic studies were obtained in all the individuals of different populations of *C. dussumieri* in population genetic analysis. According to Weising et al., (1995), candidate species-specific and population specific RAPD fragments can be converted to sequence-characterized amplified region (SCAR) markers. The species specific bands obtained in the present study can be converted to SCAR markers (Sequence Characterized Amplified Region) for precise and accurate identification of these group of fishes.

The primers used in the study were able to generate polymorphism. The percentage of polymorphism was similar for River Periyar and
Chalakudy 28.57%. For Sharavathy, the percentage of polymorphism was 32.65%. The overall value for all population taken together was 57.14%. All the selected eight primers generated polymorphism. The total number of polymorphic loci was 28 (57.14%). This result suggests that the populations have not been isolated by any barriers to gain or lose a species-specific DNA fragments. The percentage of polymorphism of *C. batrachus* populations ranged from 26.5% to 30.5% in India (Khedkar, 2010). 75% polymorphism reported by Islam et al., (2005) in *Catla catla*, 55.76% in *Oreochromis niloticus* by Zaeem and Ahmed, (2006) and 64.98% in *Mystus vittatus* by Garg et al., (2009c) is also comparable to the result obtained in the current study.

The technique of RAPD revealed minimum genetic variation between the populations of *C. dussumieri*. Average heterozygosity or gene diversity (h) is supposed to be a more appropriate measure of genetic variation rather than the proportion of polymorphic loci (Nei, 1987). The gene diversity values were almost similar in the three tested populations (0.1312, 0.1248 and 0.1437 in River Periyar, Chalakudy and Sharavathy respectively) with a value of 0.1634 in overall population. Neis unbiased genetic distance in the range of 0.1347 to 0.2876 was obtained for the population study in the yellow catfish, *Horabagrus brachysoma* by Muneer et al., (2009). Jeena, (2013) reported a gene diversity value ranging from 0.1292 to 0.1462 in *Panulirus homarus homarus* and 0.718 to 0.1375 in *Thenus unimaculatus* species.

According to Yeh, (1999), Shannon diversity Index (I) is a relative estimate of the degree of variation within each population. The Shannon Information Index (I) ranged from 0.1796 (Chalakudy) to 0.2063 (Sharavathy) with an overall value of 0.2598. A combination of genetic variability tests composed of percentage of polymorphism (P), Shannon
Information Index (I) and Nei’s gene diversity (h) conducted in the present study indicates that the genetic variation within and between populations of *C. dussumieri* tested is very less. The major reason for this reduced genetic variation may be due to the loss of natural breeding habitat as evident from the literature. It may also happen if the species is having small effective population sizes or through founder effects. The information developed from mitochondrial ATPase gene confirms the chance of recent population bottleneck or founder effects.

### 5.4.2. Genetic differentiation and gene flow

The coefficient of genetic variation (GST) value obtained for *C. dussumieri* population genetic analysis was 0.0478. Genetic differentiation is classified into four categories based on FST / GST values according to Wright (1978). The groups are 0-0.05- little genetic differentiation, 0.05-0.15- moderate genetic differentiation, and 0.15-0.25- large genetic differentiation and above 0.25 very large genetic differentiation. Based on this classification of populations, the GST value obtained in the present study indicated very less genetic differentiation among tested populations of *C. dussumieri*.

The value of gene flow (Nm) (Slatkin, 1993) is an indication whether the considered population of a species evolves as an independent unit. Theoretically value of Nm>1 is sufficient to prevent random differentiation by genetic drift (Slatkin, 1993). High gene flow obtained from the present study leads to high level of genetic mixing from the selected populations.

### 5.4.3. Genetic distance between populations

Nei’s unbiased genetic distances (Nei, 1972) are considered suitable for long evolutionary processes, with population divergences due to genetic drift and mutational events (Weir, 1990). Average pair wise similarity index
(SI) and the genetic distance (GD) based on Nei’s unbiased measures of genetic identity and genetic distances between the three populations studied were 0.9556 and 0.0454 respectively. The low genetic distance and high genetic identity is a sign of single interbreeding population, possibly with high levels of gene flow.

The deficiency of genetic differentiation between the tested populations of *C. dussumieri* is further reinforced by the UPGMA dendrogram constructed based on Nei’s (1978) genetic distances. The River Periyar and Chalakudy formed a single cluster where as Sharavathy formed another cluster. This result is supported by the geographical proximity between the populations. River Periyar and River Chalakudy are closely positioned and interconnected at lower stretches, where as River Sharavathy is away from the other two populations. Even though clustering was noticed it was supported by low bootstrap values.

Based on genetic variability, genetic differentiation, gene flow and genetic distance between the populations, it can be concluded that the three tested populations of *C. dussumieri* are homogenous. Sofia et al., (2008) reported similar results showing low polymorphism value of 28-39%, gene diversity value of 0.12-0.15 and unbiased genetic distances ranged from 0.0253 to 0.0445 indicating moderate genetic differentiation among populations of catfishes and attributed it to the eroding genetic variation to different factors related to its habitat. Low levels of gene diversity and a low level of differentiation as well as genetic structuring among populations has led to the idea of a unique homogenous population in the marine fish *Atherinella brasiliensis* (Da Silva Cortinhas et al., 2010) too. Little genetic population sub structuring has been detected with this technique in parrot fishes (Geertjes et al., 2004) leading to a conclusion of high migration rate and relatively open sub-populations.
Even though RAPD PCR is considered to be an outdated marker due to its sensitivity to several factors, it is useful, if it is used with caution. Population genetic structure of *C. dussumieri* was tested using RAPD technique in this study. Several species specific markers obtained can be used as probes. SCAR markers can be developed from these species specific markers. But, the general assumptions on which RAPD analysis are done may not always hold true in the natural environment. Utmost care should be taken while scoring of data. Combination of mitochondrial and nuclear markers was approached in this study as it is helpful in elucidating a more reliable and detailed picture of historical and present-day population structure (Palero et al., 2008; Babbucci et al., 2010) and the results were concordant also.

5.5. Implications for fisheries management and conservation

In freshwater ecosystems, if populations are depleted in one region through fishing activities then it is unlikely that they will be recolonised from adjacent geographic regions by immigration over the short to medium timescale. If overexploitation occurs within a very limited area on a local scale, then targeted populations are likely to be replenished from other proximate sub-populations. Hence a useful management strategy is the one that protects at least some subpopulations within each region in which the species is found. Only such a strategy will be likely to assist in the survival of the species and preservation of intra specific genetic diversity. Management measures that attempt to limit fishing pressure across a wide geographic area will reduce the genetic diversity of the entire regional population. Such a strategy can have disastrous consequences if control of exploitation is ineffective or quotas are set at the wrong level. Effective management of fisheries resources requires critical information on the population or stock structure of the exploited species. Therefore, the present
study has important implications for the conservation of genetic diversity in *C. dussumieri* fish populations of the Western Ghats.

Population genetic analysis of *C. dussumieri* in the selected river systems showed less genetic difference between the populations. The less genetic difference between the tested river systems is consistent with a one stock management. The very less genetic divergence seen for the species suggests that the population has undergone a rapid reduction in size in the recent evolutionary past. The reduced level of variation may be an indication of reduced genetic health of the population. The reduced genetic health will affect the long term viability of the species. Great efforts should be taken to conserve the genetic variation currently exists to decrease further drop off. Hence conservation actions should be done on *C. dussumieri* populations. A propagation assisted rehabilitation of the natural populations is advisable for the species. Protection of natural breeding habitats should be addressed very well.

These results together with observation of decline in abundance of the species along the rivers of Western Ghats in recent years (Gopi, 2000; Kurup and Radhakrishnan, 2006) reinforce the need for more studies/proposals for conservation of these habitats against overexploitation. More studies on the biology, behavior and population genetics to determine the diversity and level of gene flow between streams within the catchments of the species are needed for better understanding on the differences revealed in the present study.