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MICROSATELLITE MARKERS AND THEIR APPLICATION IN FISHERIES

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***Abstract**: Microsatellites are the most popular and versatile genetic marker having various applications in population genetics, conservation biology, and evolutionary biology. They represent an abundant source of genetic markers which are highly polymorphic and dispersed evenly throughout eukaryotic genomes. Genetic variation arises between individuals leading to differentiation at the level of population, species and higher order taxonomic groups. Development of microsatellite markers has powerful ability to detect genetic variations between individuals, populations or species. Microsatellites represent ideal molecular markers as they are co-dominant in nature, highly polymorphic, easily typed, and Mendelian inherited, all properties which make them very suitable for the study of population structure and pedigree analysis. These properties make microsatellites an ideal genetic marker for conservation genetics and fisheries management These microsatellite markers combined with new statistical developments have revolutionized the analytical power, necessary to explore the genetic diversity and have become the markers of choice because of their wide range of application in population genetic, gene tagging, pedigree analysis, conservation and evolutionary biology.*

Keywords: Microsatellite markers, Fisheries, Genetic diversity analysis

INTRODUCTION

Of all the animals and plants in the aquatic environment, fish is the most important source of human food. It constitutes the main part of the diet in many cultures and is also a major source of dietary protein for many communities living in the coastal belt or near some other water source like rivers, lakes, reservoirs, etc.. Along with rich content of protein, fishes are also a good source of PUFAs (Poly Unsaturated Fatty Acids) like ω-3. Fish and fisheries not only provide a significant portion of the protein available for human consumption, but they are also an economically significant activity, providing jobs and investment opportunities to a large mass of people who are directly or indirectly linked with this field. Not only this, fisheries have been an important means of improving the balance of international trade for many countries. Most of the fishes used for human consumption are obtained through exploitation of wild populations. With this increasing exploitation, there has been a need of proper management of these populations. The management of the wild populations comprising commercial or sport fisheries presents various problems of which genetic problems are unique to fisheries management.

Genetic variation in populations became a subject of scientific enquiry in the late nineteenth century prior even to the rediscovery of Mendel's paper in 1900. Genetic variation, in the form of multiple alleles of many genes, exists in most natural populations. All organisms are incessantly undergoing micro- and macro- evolutionary processes both at molecular and organism levels. In fact, the process of evolution starts at the molecular level, more precisely from a single base of the DNA molecule, and ends up in variations at the organism level (Abdul-Muneer, P. M., 2014) and causes

some mutations because of normal cellular operations or interactions with the environment, leading to genetic variation (polymorphism). These variations that happen to the genes in turn produce individuals, which are different either at the molecular level or at the organism level. These individuals may form separate groups within the species itself (known as "stocks") and such groups are the fundamental genetic units of evolution.

In most sexually reproducing populations, no two organisms (barring identical twins or other multiple identical births) can be expected to have the same genotype for all genes (Hartl and Clark, 1997). Genetic variation in a species enhances the capability of organism to adapt to changing environment and is necessary for survival of the species (Fisher, 1930). In conjunction with other evolutionary forces like selection and genetic drift, genetic variation arises between individuals leading to differentiation at the level of population, species and higher order taxonomic groups. Reduction in the genetic resources of natural fish populations has become an important fisheries management problem. Much of the reduction is due to various human activities. Not only the genetic diversity of many fish populations has been altered, but many thousands of populations and species have been extirpated by pollution due to over fishing exploitation, destruction of habitat, blockage of migration routes and other human developments (Ferguson, 1995). Reduction of genetic resources in fish is part of a larger global concern for the genetic resources of the biosphere. For this reason, molecular genetic research should be strongly supported, for it is vital to the long-term management of fisheries resources (Park and Moran, 1995).

In fisheries biology, the detection of genetic variation among individuals is achieved by the applications of molecular markers. Molecular markers are powerful tools for the analysis of genetic biodiversity, which are based on DNA sequence polymorphisms. According to technical principles, there are three classes of molecular markers:

1. **Nucleic acid hybridization based on complementary bases**, e.g., restriction fragment length polymorphisms (RFLPs),
2. **Polymerase Chain Reaction (PCR) based on DNA amplification**, e.g., random amplification of polymorphic DNAs (RAPD), amplified fragment length polymorphisms (AFLP), microsatellites or simple sequence repeats (SSRs) and
3. **Single Nucleotide Polymorphisms (SNPs)**.

Modern sequence based marker systems for genetic analysis such as Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs) are now predominantly used (Duran et al. 2009). The first technique, RFLP, has been decreasingly used due to the difficulties involved in manipulating high throughput sampling but the third technique, SNPs, represents high costs related to large-scale genotyping. However, the cost-effective PCR-based techniques have been largely used.

Nowadays microsatellites have become the marker of choice for application in fish population genetic studies (Beckmann and Soller, 1990). The most common use of molecular markers in fisheries biology is to determine if samples from culture facilities or natural populations are genetically differentiated from each other. They are also used to identify different species in the event of taxonomic disputes and to detect genetic introgression in a species. A common objective of molecular genetics analysis is to

diagnose the differences among presumed stocks in either nuclear allelic types or mtDNA haplotypes (Danzmann and Ihssen, 1995).

MICROSATELLITE MARKERS

The advent of PCR coupled with automated DNA sequencers made feasible major technological innovations such as assessment of the variations at microsatellite loci (Weber and May, 1989). Microsatellites were detected in eukaryote genomes almost thirty years ago and they are the most promising PCR-based markers. Microsatellites are tandemly repeated motifs of variable lengths that are distributed throughout the eukaryotic nuclear genome in both coding and non-coding regions (Jarne & Lagoda, 1996). They are short tandemly arrayed di-, tri-, or tetra- nucleotide repeat sequences with repeat size of 1–6 bp repeated several times flanked by regions of non-repetitive unique DNA sequences (Litt & Luty, 1989 and Hecker *et al.*, 1999). They are also called as "Simple Sequence Repeats" (SSR) (Tautz, D., 1989) or "short tandem repeat" (STR) DNA by Edwards et al. (1991). Microsatellite motifs have been estimated to occur as often as once every 10 kb in fishes. They have been found inside gene coding regions, introns and in the non-gene sequences (Liu et al., 2004). Different alleles at a locus are characterized by different number of repeat units but same flanking region (Fig. 1). Alleles at microsatellite loci from small samples of genomic DNA can be amplified by the polymerase chain reaction (Saiki *et al.*, 1988). Polymorphism at microsatellite loci was first demonstrated by Tautz in 1989.

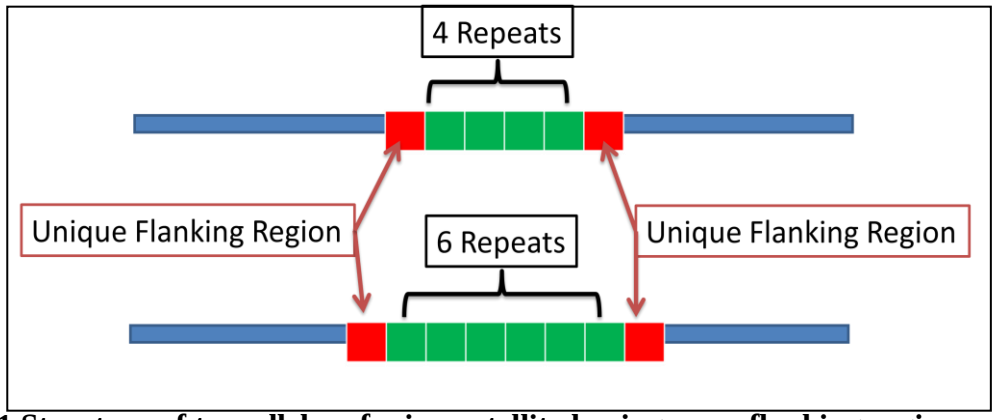


Fig. 1 Structure of two alleles of microsatellite having same flanking region

Due to the high mutation rate of microsatellites, they are potentially the most informative molecular marker with the advantage of easy and low-cost detection by PCR. Microsatellites are also co-dominant markers (i.e., identify all the alleles of given locus and also trace homozygosity and heterozygosity in genotype) and have been inherited in a Mendelian fashion. They were found to be informative in several species which showed almost no variation at other markers (Taylor *et al.*, 1994). This is the reason they have become the markers of choice for a wide range of applications in population genetic, conservation and evolutionary biology and represent ideal molecular markers because they have multiple alleles which are highly polymorphic among individuals (Mojekwu and Anumudu, 2013).

Types of Microsatellite Markers

According to the type of repeated sequence presented, microsatellites have been classified as:

1. Perfect, when showing only perfect repetitions, e.g., $(AT)_{20}$,
2. Imperfect repeats, when the repeated sequence is interrupted by different nucleotides that are not repeated, e.g., $(AT)_{12}GC(AT)_8$ and
3. Composite, when there are two or more different motifs in tandem, e.g., $(AT)_7(GC)_6$.

The composite repeats can be perfect or imperfect. The sequences of di-, tri- and tetra-nucleotide repeats are the most common choices for molecular genetic studies (Selkoe and Toonen, 2006).

Based on the length of repeat motif, microsatellites can be of two types (Temnykh et al., 2001):

1. Class I microsatellites- perfect SSRs of ≥20 nucleotides in length.

2. Class II microsatellites- perfect SSRs of ≥12 nucleotides and <20 nucleotides in length.

Advantages of Microsatellite Markers over Other Markers

The high variability exhibited by microsatellite loci relies on their mutation rate, several orders of magnitude higher than base substitution rates. Figures in the range of 10^{-3} to 10^{-4} per locus and generation have been usually reported for microsatellite loci (Ellegren, 2000; Schlötterer, 2000). Microsatellites are more variable and informative than RFLP, RAPD (He *et al.*, 2003) and AFLPs (Lee *et al.*, 2004). The technique is PCR-based, thus require only low quantities of template DNA (Kumar *et al.*, 2009; Wolko *et al.*, 2010). The application of lengthy primers and high annealing temperatures during genotyping enhances reproducibility. The ability to use more than one set of optimized SSR markers in a single reaction (multiplexing of markers) significantly reduces the analytical costs involved in genome analysis. They are also useful for parentage analysis and for estimating the degree of relatedness of individuals or groups. Multiallellic microsatellites are considered to be the best marker system for the detection of intervarietal polymorphisms (Stepien *et al.*, 2007). They offer wide applications in the preparation of genome-wide genetic maps and comparative

mapping.The major advantages of microsatellite markers are co-dominant transmission (the heterozygotes can be distinguished from homozygotes), locus-specific in nature, highly polymorphic and hypervariable, high information content and providing considerable pattern, relative abundance with uniform genome coverage, higher mutation rate than standard, and easy to sample preparation. The presence of uninterrupted stretches of identical repeat units, short size range, and availability of statistical procedures for inter-population comparisons and their abundance in fish genomes have resulted in abundant use of microsatellite in fisheries.

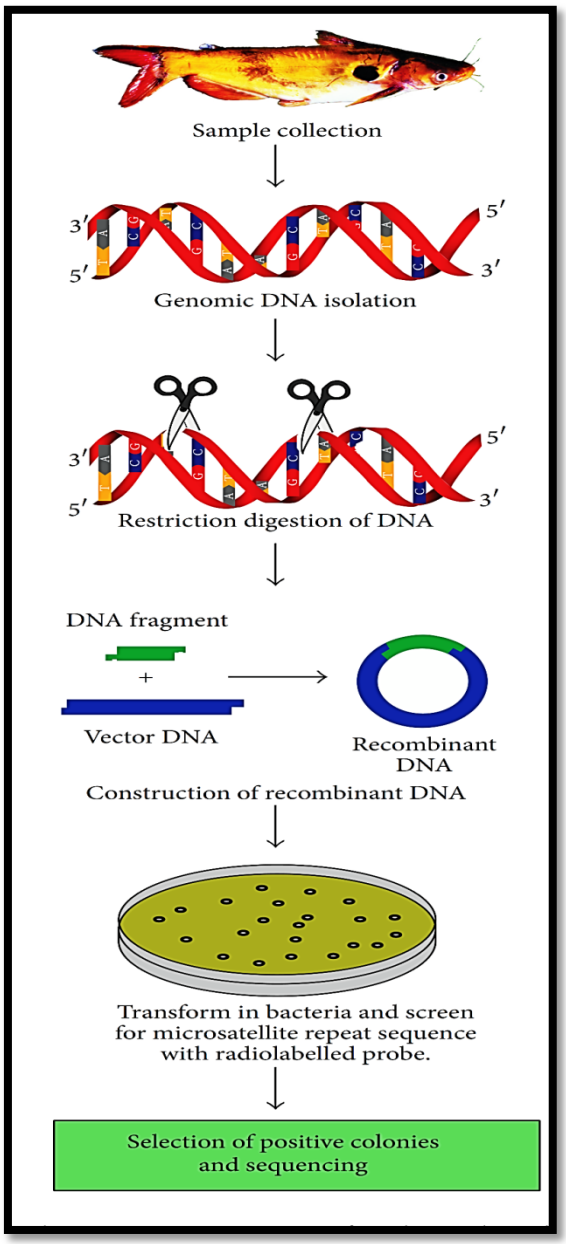
Development of Microsatellite Markers

In traditional method of development of microsatellite markers (Fig. 2), the genomic DNA was restricted, ligated into suitable vectors and transformed to generate a non-enriched genomic DNA library. Clones were then spotted onto gridded nylon filters and screened with radio-labelled SSR probes or subjected to enrichment with 'biotin labelled probes-streptavidin capture system' and sequenced. Cloning of DNA fragments prior to enrichment steps makes it ideal to screen for a wide range of SSR motifs and reduce/ avoid redundancy.(Senan, S., *et al.,* 2014) These microsatellite markers developed are species specific maekers. But the high cost of developing species-specific markers has been the main challenge of microsatellite markers in the past is the (Castoe *et al.*, 2010). However, it is reported that the flanking region/sequences of any microsatellite markers exhibit slower mutation rate than SSR region (Holmen *et al.*, 2009), permitting their sequence conservation across species or genera. This homology allows the amplification of primers designed for one species to other members of the

same species or genera (cross-species/ cross-genera amplification or transferability). Transferability offers potential for the low cost generation of microsatellite markers for related or distant species. This information led to development of microsatellite markers by modern technique known as cross species amplification which is shown below by a flow diagram (Fig. 3) and, this has been further alleviated with the advent of next-generation sequencing, which allows the detection and characterization of SSR loci easily achievable with simple bioinformatics.

APPLICATIONS OF MICROSATELLITE MARKERS IN FISHERIES

Microsatellites are the most popular and versatile genetic marker with various applications in conservation biology, evolutionary biology and population genetics. Microsatellites are co-dominant in nature, highly polymorphic, easily typed, and Mendelian inherited, all properties which make them very suitable for the study of population structure and pedigree analysis and capable of detecting differences among closely related species (Danish *et al.*, 2015).



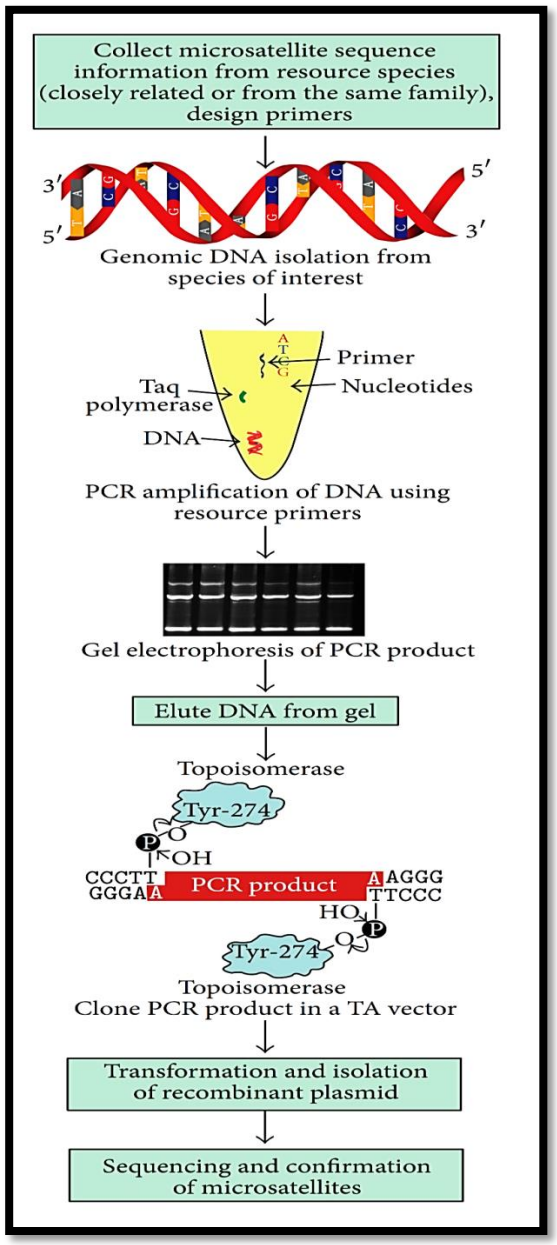


Fig. 2 Systematic representation of traditional method of development of species specific microsatellite markers		**Fig. 3 Systematic representation of development of microsatellite markers for fishes using cross species amplification**

(*Source:* Abdul-Muneer, P. M., 2014**)**

Some microsatellite loci have very high numbers of alleles per locus (>20), making them very useful for applications such as parent-offspring identification in mixed populations, while others have lower numbers of alleles and may be more suited for

population genetics and phylogeny Fig 4 (Al-Atiyat *et al*., 2012). Primers developed for one species will often cross-amplify microsatellite loci in closely related species (Boris *et al*., 2011). Genotyping of microsatellite markers is usually straightforward (Castoe *et al*., 2010). By reason of the widespread abundance of microsatellites in genomes and their relative uniform distribution, mutation dynamics and high degree of polymorphism, microsatellites have emerged as valuable tool in genetic mapping, forensic identity testing and population studies.

Microsatellite markers provide us vital information about the fish population in various ways. There are various applications of microsatellite markers in fisheries *viz.* in population genetic analysis, in detection of parentage and pedigree analysis, in genome mapping, in conservation genetics, in detecting demographic bottlenecks, in identification of genetic variability between and within stocks, monitoring genetic changes in stocks (gene tags), in detection of quantitative trait loci (QTL) (Fjalestad *et al*., 2003; Subasinghe *et al*., 2003; Chistiakov *et al*., 2005), some of which are discussed below:

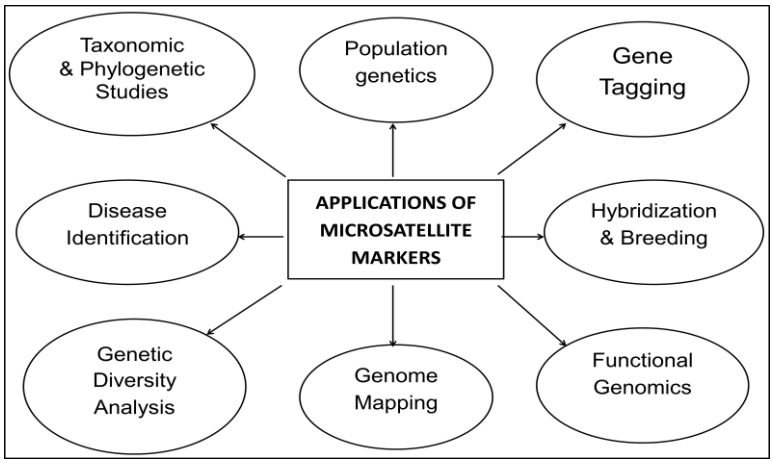


Fig. 4 Various applications of microsatellite markers in fisheries

Microsatellite markers in population genetic, parentage and pedigree analysis

Microsatellites are one of the best suitable genetic markers for analyzing pedigree, genome variation, population structure, evolutionary process and fingerprinting purposes (Danish, *et al.,* 2015). Diverse studies generally show that high allelic variation enables the use of microsatellites as a versatile molecular marker in population genetics. Population genetics analyses can provide data on a variety of important evolutionary parameters, including standing levels of genetic variation, the partitioning of this variability within/between populations, overall levels of inbreeding, effective population sizes and the dynamics of recent population bottlenecks (Ellis, *et al.,* 2007). Brown, *et al.,* (2003) has demonstrated nuclear microsatellite and mitochondrial marker estimates of population structure in striped bass (*Morone saxatilis*) and found that this highly migratory anadromous and long-lived fish has recently recovered from a severe decline in population size. Adams and Hutchings, 2003, assessed the genetic variation using microsatellite genetic markers to identify the population structure of brook charr, *Salvelinus fontinalis.* Similarly, population genetic studies and parentage assignment in cobia, an important recreational fishery in the south-eastern United States, was performed by Pruett, *et al.,* 2005. Population structure of sockeye salmon (Nelson, *et al.*, 2003) and Chinook salmon (Beacham, *et al.*, 2003) has also been examined using microsatellite markers.

Microsatellite markers proved to be a useful tool for studying both population genetics (e.g. stock structure, effective population size) and inheritance of traits important to aquaculture. They are best suited marker used in aquaculture for parentage and pedigree

analysis in selective breeding programmes as they solve the main problem of this programme which is to tag the newly born individuals to separate one family from other which is not possible to do with other physical tags due to small size of the individuals. Hence, more families can be kept in the breeding stock without the need for using separate tanks at early ages and microsatellites have been used to assess family/parentage identification in many species and can be used to discriminate fish in mixed family groups (Cross, *et al*., 2004; Duran, *et al*., 2009; Boris, *et al*., 2011; Al-Atiyat, *et al*., 2012).

Microsatellite-based traceability methods have also confirmed to be very useful for a precise acquisition of pedigree information in fish species (Castro, *et al*., 2006; Liu and Cordes, 2004). The broad areas of applications of microsatellite markers are depicted in the development of polymorphic microsatellite markers to determine the population structure of the Patagonian toothfish, *Dissostichus eleginoides*, has been reported by (Rogers, *et al.*, 2006). Similarly, Appleyard *et al.*, (2002) examined seven microsatellite loci in the same species of Patagonian toothfish from three locations in the Southern Ocean. Similarly, several authors reported population genetic structure of different species of catfish; few of them are in the farmed catfish from Tamaulipas, Mexico (Perales-Flores, *et al.*, 2007), in neotropical catfish (Ribolli, *et al.*, 2012) and in *Pseudoplatystoma reticulatum* (De Abreu *et al.*, 2009). Microsatellite markers have also been used to evaluate the genetic diversity of six populations of red hybrid tilapia from different farms of Colombia by Briñez, *et al*, in 2011. Singh, *et al*., (2012) assessed genetic variation in samples of *Labeo calbasu* from twelve riverine locations across India using nine polymorphic microsatellite loci and the study provided conclusive

evidence about the distinct population substructure and gene pool of *L. calbasu* in different rivers in India. The estimates also pointed out that population in some of these rivers have undergone genetic bottleneck. Many others have also reported studies of polymorphic microsatellite loci to evaluate population structure of different fish species.

1) Microsatellite markers in genome mapping

The most common use of genetic markers in fisheries biology is to determine if samples from culture facilities or natural populations are genetically differentiated from each other. They provide a novel fingerprinting approach applicable for taxonomic and phylogenetic comparisons and as a mapping tool in a wide range of organisms (Zietkiewicz, *et al.*, 1994). In 1998, Kocher, *et al.* constructed a genetic linkage map of tilapia (*Oreochromis niloticus*) using 62 microsatellite markers. The map spans 704 Kosambi cM in 30 linkage groups covering the 22 chromosomes of this species. Twenty-four of these linkage groups contain at least one microsatellite polymorphism. Thai, *et al.*, (2007) also reported that Vietnam wild common carp populations exhibited more genetic diversity than cultured populations in term of allele richness and observed heterozygosity. They are also used to identify different species in the event of taxonomic disputes and to detect genetic introgression in a species. According to Zhan, *et al*., 2009, microsatellite analysis has been very successful in detecting the genetic impact of culture. The higher sensitivity of microsatellite to phenomena such as genetic drift and founder effect make it ideal for monitoring the consequences of founding and propagation in aquaculture than of mtDNA and allozyme (Duran, *et al*., 2009).

SSR markers have important applications in monitoring inbreeding depression. Fishback, *et al*., in 1999 successfully used microsatellite markers for minimizing

inbreeding in rainbow trout. Microsatellites with only a few alleles are well suited for population genetic studies, while the more variable loci are ideal for genome mapping and pedigree analysis and the fixed or less polymorphic microsatellite loci are used to resolve taxonomic ambiguity in different taxa. In 2013, Ceyhun and Çiltaş, investigated the genetic variation among five populations of *Salmo trutta* sp. L., indigenous to Turkey, constituting two populations from lakes and three from rivers and observed that the genetic distance was shorter in the samples collected from lakes as compared to that of the rivers. Bulasag, *et al.*, (2015) constructed microsatellite enriched libraries for *Glossobius giuis* and *Rhinogobius giurinus* using BLAST analysis and cross-amplified it in other goby species which showed positive microsatellite in all species. Napora-Rutkowski, *et al.*, 2017, using microsatellite markers, AFLP and mtDNA, genetically characterized 20 common carp strains bred in Poland and compared it with the population of common carp from that in Japan. The microsatellite analysis of the studied strains showed moderate genetic variations between the strains and Bayesian clustering analysis of microsatellite loci was also done which divided the strains into 17 distinct clusters. Seyoum, *et al.*, 2016, isolated 24 polymorphic microsatellite markers from sheepshead (*Archosargus probatocephalus*) and screened in 57 specimens from the Indian River, Florida. By the results of this study, it was reported that the polymorphic markers used in this study can be used to search for genetic evidence for the morphologically defined subspecies, to elucidate the fine-scale genetic population structure of this broadly distributed coastal species, and to provide an opportunity to directly compare results of population delineation between nonspecific and species-specific markers.

2) Microsatellite markers in detection of quantitative trait loci (QTL)

Microsatellites are one of the best suitable genetic markers for analyzing pedigree, genome variation, population structure, evolutionary process and fingerprinting purposes (Danish, *et al.*, 2015). Highly polymorphic microsatellite markers have great potential utility as genetic tags for use in aquaculture and fisheries biology. They are powerful DNA markers for quantifying genetic variations within and between populations of species (Muneer, P.A. *et al.*, 2009). The qualities of microsatellites make them very useful as genetic markers for studies of population differentiation and stock identification (Liu and Cordes, 2004), in kinship and parentage exclusion (Webster & Reichart, 2005 and Hansen, *et al.*, 2001) and in genome mapping (Sanetra, *et al.*, 2009). Microsatellites are also being used as genetic markers for identification of population structure, genome mapping, pedigree analysis, and to resolve taxonomic ambiguities in many other animals besides fishes (Lu, X. *et al.*, 2014; Nikbakht, *et al.*, 2013; Arias-P´erez, *et al.*, 2012; Fernandes, *et al.*, 2012; Upadhyay, *et al.*, 2012; Joshi, *et al.*, 2012; Xu & Liu, 2011 and Supungul, *et al.*, 2000). The analysis of the DNA sequences surrounding the trait linked markers also allows identification of genes representing QTL (Quantitative Trait Loci).

3) Microsatellite markers in conservation genetics

Endangered species have small and declining populations, so loss of genetic diversity and inbreeding are unavoidable in them. Since inbreeding reduces reproduction and survival rates and loss of genetic diversity reduces the ability of populations to evolve to cope with environmental changes. In small populations of threatened species, these genetic factors can contribute to the risk of extinction (Frankham, 2003). Microsatellite

markers allow the assessment of genetic structure, dispersal and demographic resilience in a particular studied species as a function of environmental change and variation, thereby aiding freshwater monitoring and conservation. Macdonald, *et al.,* 2016 used next generation sequencing technique for developing microsatellite markers to enable conservation genetic investigation of various widespread aquatic invertebrate species. Antoro, *et al.,* in 2006, studied the genetic diversity among populations of orange spotted grouper collected from six sites from Thailand and Indonesia and found that despite of enhanced substantial gene flow, the level of heterozygosity of this specie was relatively low as compared to other migratory fishes. Recently, Larsen, *et al.*, (2011) showed differences in salinity tolerance and its gene expression in two populations of Atlantic cod (*Gadus morhua*). Drinan, *et al.,* (2011) reported 20 microsatellites for determining the patterns of population genetic variation in westlope cutthroat trout, *Oncorhynchus clarkia* lewisii in 25 populations from four rivers. Davies, *et al.*, (2011) identified 12 microsatellite loci in tuna species of genus *Thunnus* and investigated genetic polymorphism at these loci in North Atlantic and Mediterranean Sea populations.

In 2013, Qin, *et al.,* examined the genetic diversity and population structure to evaluate the germplasm resource of *Pampus argenteus* form three different sites using 13 microsatellite markers and found a high level of genetic diversity within all the populations. Chen, *et al.,* (2012) analyzed genetic variation in four grass carp populations, three of which were introduced population and one native of China, and found that allelic richness and heterozygosity was lower in introduced population as compared to native which was presumed to be happened due to small founder

population. Introduced species often experience founder effects and show reduced genetic diversity within populations, as well as increased isolation among populations (Bakker, *et al*., 2009; Dlugosch and Parker 2008; Henry, *et al*., 2009; Wang, *et al*., 2009). On the other hand, a high level of genetic diversity has been found in many introduced populations as a direct result of multiple introductions that may lead to large amounts of genetic variation and novel genetic combinations in a population (Rosenthal, *et al.*, 2008; Andreakis, *et al.*, 2009 and Chun, *et al*., 2009). Rapid population expansion may mitigate the loss of genetic diversity or help maintain substantial genetic variation within population after introduction (Zenger, *et al*., 2003). DeWoody and Avise (2000) reported microsatellite genetic variation in marine, fresh water, and anadromous fishes compared with other animals. The adaptive evolution of an introduced population in new habitats is often accompanied by genetic change, and results in successful species colonization and invasion (Phillips *et al*., 2006; Prentis *et al*,. 2008).

Previously, Salzburger, *et al.* (2002) reported a case of introgressive hybridization between an ancient and genetically distinct cichlid species in Lake Tanganyika that led to the recognition of a new species. Faulks and Östman, in 2016, assessed the microsatellite genetic diversity and population structure of three salmonid species of which two were native (Arctic charr and Brown trout) and one introduced (Brook trout) to aid in formulating appropriate conservation management plans. The results showed a high level of genetic diversity in all three populations but brown trout and brook trout also showed elevated inbreeding coefficients. Also in all three populations, genetic differentiation was seen at only a few specific locations.

Baillie, *et al.*, (2016) used microsatellite markers to monitor the genetic and phenotypic diversity of lake trout *Salvelinus namaycush* in Lake Superior. The comparison of data sets from year 2004-2013 with that from 1995-1999 revealed a substantial loss in genetic diversity of this fish which was related with the historical fish harvest exacerbated by intensive stocking and invasion of non-native species which led to overlap in feeding and breeding ground of this fish. In 2016, Wegleitner, *et al.*, were able to trace down the site of occurrence and introduction of *Channa argus*, an invasive species spread all over the Hudson River and Chesapeake Bay, to River Potomac and were also able to get the evidence of multiple introductions into the U.S. waters and human mediated secondary spread from these founder populations.

Das, *et al.* (2005) and Gopalakrishnan, *et al.* (2009) carried out characterization of dinucleotide microsatellite repeats in *Labeo rohita*. As these factors would lead to a reduction in reproductive fitness efforts to increase the genetic diversity of the fish species should be given high priority for conservation of the species (Padhi and Mandal, 2000). The natural populations of the endangered species can be enhanced by "supportive breeding." In this program, a fraction of the wild parents are bred in captivity and the progeny are released in natural waters.

4) Microsatellite markers in aquaculture and comparing the genetic variation between wild and cultured populations

Microsatellite markers also find application in aquaculture to assess loss of genetic variation in hatcheries through comparison of variation estimates between hatchery stocks and wild counterparts. The information is useful obtained in monitoring farmed stocks against inbreeding loss and to plan genetic up gradation programmes. A major

aspect such studies address is concerned with the assessment of farm escapes into the natural population and introgression of wild genome. All wild-unstocked samples were highly differentiated populations and significantly different from each other and from hatchery samples. Genetic diversity was investigated using microsatellites between farmed and wild populations of Atlantic salmon (Norris, *et al.*, 1999). Farmed salmon showed less genetic variability than natural source population in terms of allelic diversity. Variation in allozymes and three microsatellite loci was assessed in populations of wild and cultured stocks of *Sparus aurata* (Palma, *et al.*, 2001 and Alarcon, *et al.*, 2004). The microsatellite heterozygosity values were high in wild, but lower in the cultured samples.

Due to declining population of pacific threadfin in Hawaii, Pan and Yang (2010) used six microsatellite DNA markers on two populations of the specie for monitoring of this population and the results indicated no significant genetic differentiation between wild and hatchery population. An, *et al.*, 2014, studied the genetic diversity and population structure of four wild and three hatchery stocks of *Palatichthys stellatus* using nine microsatellite markers and found high degree of polymorphism within all populations but a reduced genetic diversity was seen within the cultured stocks when compared with the wild stocks. The level of distribution of genetic variation between wild and hatchery bred population of *Stephanolepis cirrhifer* was also investigated by An, *et al*, (2011) using 10 microsatellite markers and the results showed that the genetic variability was similar in both the populations and the genetic differentiation was very much significant.

Earlier in 2008, Liu, *et al.*, performed genetic diversity analysis of four wild and one hatchery samples of *Cynoglossus semilaevis* using 15 ISSR primers and reported that the hatchery sample showed least genetic variability among all five samples under study. Sahoo, *et al.*, in 2014, assessed the genetic variation among three four populations of *Labeo rohita* using eleven microsatellite primers. Out of these four populations, three populations were of three river systems of India (*i.e.*, Mahanadi, Godavari and Krishna) and one of culture system. They noted that the highest genetic differentiation was observed between Godavari and cultured population and least between Godavari and Krishna river population., For effective brood-stock management and conservation, Sultana, *et al.*, 2015, used five species specific microsatellite markers to assess the genetic variability and level of inbreeding among different populations of *Labeo rohita* collected from six different hatcheries.

Ozerov, *et al.*, (2016) assessed the genetic impact of inadvertent gene flow due to hatchery release of Atlantic salmon on wild population in Gulf of Finland using 17 microsatellite markers and 1986 SNPs and found a congruent population genetic structuring that indicated genetic introgression which had eventually changed the genetic makeup of wild population by increasing genetic diversity and reducing genetic divergence. In 2016, Mirimin, *et al.*, also used microsatellite markers to evaluate the level of genetic diversity and population structuring of *Argyrosomus japonicas* along South African coast and indicated the adverse effects of high fishing pressure by noting low and declining trends of effective population size, weak genetic differentiation among samples and signatures of bottleneck. They were also able to trace the parentage of this specie from the individuals from commercial hatchery and discrimination

between farmed and wild population. Thus, microsatellite markers have wide range of applications in all population genetics, fisheries management and aquaculture practices.

CONCLUSION

Microsatellite markers are very powerful tool for identifying fish stock structure and pedigree analysis and study of genetic variation in closely related species. They also help in estimating the differences in the allelic frequencies among different populations which enable the application of genetic stock identification models to determine the contribution of individual stocks. Thus, these different size modes could indicate some sort of genetic heterogeneity within the population. Analysis based on microsatellite markers provides essential information for formulating meaningful conservation strategies for fisheries and aquaculture. This along with the other technologies like captive breeding and sperm cryopreservation can be integrated into a package for conserving genetic diversity and rehabilitation of the natural populations of fish species. This review has brought forth available evidences to suggest that the detection of microsatellite variation plays an important role in the study of population genetics and evolution. Genetic studies can show the fundamental reproductive units of species and require fisheries management policies to take this population structure into account. The management of fish stocks should be based on each population and therefore should be harvested and treated separately in research as well as management policy. Maintaining the maximum level of genetic variations in stocks is vital for the preservation of genetic resources. Microsatellites as molecular markers in molecular-ecology, population genetics and genetic-mapping are actively utilized for the conservation of threatened

species in a population by comparing the level of variability in microsatellite loci. Microsatellites have thus become an important tool for fisheries researchers working in the field of ecology, population biology and conservation planning.

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