Pattern and Dynamics of Genetic Stock of Freshwater Fishes using Various Molecular Markers for Their Conservation Management Concerns in the Indian Riverine System

ABSTRACT

India has a world's richest, most abundant and most promising inland fisheries resources with abundant fishes in tributaries, streams, canals, lakes, ponds, and reservoirs. There are several significant river systems in India, including the Cauvery, Tapi, Narmada, Krishna, Indus, Brahmaputra, Ganga, Mahanadi, and Godavari which showing shrinkage of fish fauna because of environmental disturbance, human interference and human threats. In order to conserve the fish diversity, the molecular markers are helpful in determining genetic diversity, gene polymorphism and gene flows from generation to generation. The topic experienced a surge in interest due to the introduction of strong statistical analysis tools and the accessibility of rapid DNA fingerprinting, DNA sequencing techniques and consequently population genetic studies. Some molecular markers, Microsatellite markers, RAPD, Allozymes and mitochondrial (*cox1*, *cytob*, *ATPase6/8*) implications have been discussed in this review which will provide an overview of the various molecular markers that have been used by scientists to studied population genetic structure and genetic variations at various levels.

Keywords

Genetic diversity, gene diversity, gene flow, *cyto b*, *ATPase6/8*, *cox1*, haplotype, nucleotide, AMOVA, truss analysis.

Introduction

India has some of the wealthiest, most extensive, and most promising inland fishing resources in the world. India ranks ninth in the term of freshwater mega biodiversity. When it comes to biodiversity, fish are the most prominent and reliable bio-indicators of the environment. Because of numerous environmental factors, biodiversity documentation has grown in importance as a scientific endeavor in recent years (Lakra *et al*., 2011). One of the largest issues facing contemporary society is the loss of biodiversity, as the depletion of genetic resources and habitats is a growing indicator of environmental disaster (Garg and Mishra, 2023). A species genetic variability and stock structure must be known in advance for scientific

management, genetic improvement programs to prevent population decline in the species native habitat and insure the sustainability of its resources (Sah *et al*., 2020). DNA markers are essential tools that have been utilized to assess genetic diversity levels and trends in several cultured fish species (Liu and Cordes2004). The high rate of mutation in mitochondrial DNA makes it a good marker system for identifying recent population isolation, and this is only one of the many features that make it one of the most widely used DNA markers now accessible. Because nuclear DNA lacks haploid and introns, mitochondrial DNA from animals is the most effective method for species identification and analysis (Sajjad *et al*., 2023).The analysis of mitochondrial DNA (mtDNA) is a valuable tool for studying variation at both the intraspecific and interspecific levels. Its compact organization, absence of recombination, and higher rate of evolution when compared with nuclear DNA makes it exclusively important identifying genetic structure (Vidya *et al*., 2017). Within the aquaculture community, allozymes, mtDNA, RAPD, AFLP, microsatellite, SNP, and EST markers are often used genetic markers (Fig. 1). The use of DNA markers has sped up research on genetic variability and inbreeding in aquaculture, parentage determination, species and strain identification, and the creation of high-resolution genetic linkage maps for aquaculture species (Liu and Cordes 2004). This review provides an overview of several molecular markers that scientists use to study fish genetic variation in order to manage fish conservation in Indian rivers.

Taxonomic Validation using Truss analysis

The "truss network technique" is a robust statistical analysis-based tool that is validated by landmarks and employed widely by numerous workers for species discrimination (Corti *et al*., 1988).To generate the morphometric variables collected from digital photographs of specimens taken from the study areas, a truss network is constructed by linking the landmarks (Chandran *et al*., 2022), with the help of these points, the individual fish body shape can be analyzed (Verma *et al*., 2014). To identify morphometric changes, the principal component analysis (PCA), discriminant function analysis (DFA), and cluster analysis (CA) is used. Sampled specimens were laid out on a flat surface on laminated paper, which served to standardize the digital picture coordinates. Every fish sample is identified with a unique code. The digital photos are taken with a digital camera. Every fish specimen is arranged laterally on its right side, with its fins and body posture gently pushed into their optimal alignment. Fish specimen images were taken and then imported into the computer for additional processing (Gupta *et al*.,

2018). tpsUtil, tpsDig 2 v2.1, and PAST were the software platforms used in combination to extract the truss distances from the digital photos of the specimens. The program tpsUtil transforms JPEG images into tps format. The locations of the landmarks are selected according to the following criteria: 1. Reliability in terms of correspondence between specimens, 2. The ability to best describe the geometry of the form under study (Gupta 59-61). Findings of multiple research indicate that the truss technique can be useful in resolving taxonomic uncertainty by measuring variations in shape.

Species Identification by DNA Barcoding

The identification and removal of multiple taxonomic obstacles have been successfully accomplished with the use of DNA barcodes (Hebert *et al*., 2003a). The resolution of this diversity can be aided by the mitochondrial gene cytochrome-c oxidase subunit-1 (Hebert *et al*., 2003b). Although prior studies has confirmed that *COI* Sequences can accurately describe species within specific taxonomic categories, The barcode sequences of all unknown species are compared to known sequences found in a barcode sequence library. A specimen is easily identified if its barcode sequence closely resembles one of the previously recognized species that are listed in the barcode library; if not, the specimen adds a new record to the library and acts as a baseline for subsequent sequences from the same collection (Shelake *et al*., 2021). In order to barcode fish DNA, particularly that of *C. arel*, the mitochondrial *COI* and *16S rRNA* genes has been used (Soman *et al*., 2020). Typically, DNA barcoding involves a part of the mitochondrial gene cytochrome oxidase-c subunit-1 (*COX1* or *COI*) which is a high evolutionary rate. The *COI* gene amplified by PCR in *C. lingua* in Chandipur coast of Odisha had a size of about 655 bp has been used to identify the species (Jena *et al*., 2023). Gene diversity and genetic differentiation among within population of *R. gogra* were done with the help of K2P distance and Neighbour joining tree software's (Garg and Dohre, 2023). 10 barcodes were obtained for 10 species of fin fishes belonging to 9 genera 6 families (Engraulidae, Ambassidae, Teraponidae, Lutjanidae, Eleotridae and Gobiidae form Krishna river estuarine region, Andhra Pradesh, India by DNA barcoding using *COI* gene (Krishna *et al*., 2012).

Evaluation of Genetic Diversity

A particular species' unique members are not genetically similar. They have somewhat different DNA sequences, and these variations make up a species' genetic variety, or polymorphism (Ellegren and Galtier 2016). Founder stock selection in breeding programs is based on the genetic heterogeneity of natural stock. In order to establish genetic improvement programs, effectively manage natural genetic resources, and conserve them, it would be beneficial to comprehend genetic variability data (Das *et al*., 2018). To determine the phylogenetic relationships and intraspecific and interspecific genetic diversity using the mtDNA *cyt b* gene, twelve *Puntius* species were collected from eight Indian rivers. A range of 0.001 to 0.005 was calculated for the average intra-species sequence diversity, and 0.071 to 0.235 was estimated for the average interspecies sequence diversity. Between *P. chelynoides* and *P. fasciatus*, there was the most interspecies sequence diversity (0.235), while between *P. chola* and *P. sophare*, there was the least sequence divergence (0.071). Nucleotide differences within species ranged from 1 to 5, whereas the estimated average nucleotide differences were between 74 and 216. To overcome species discrimination among the populations, it is importance to estimate genetic divergence which can be done using the *cyt b* gene (Pallavi *et al*., 2012).

From each of the five individuals gathered for this investigation, the 5' partial segment of the 614 bp *E. tetradactylum COI* gene was amplified, sequenced, and 25 Genbank sequences were examined. A, T, G, and C had respective average contents of 22.6%, 29.1%, 19.2%, and 29.1%. There were no insertions or deletions discovered, and 18 haplotypes were identified from the 30 sequences (Thirumaraiselvi and Thangaraj 2015**).** Ten polymorphic allozymes and eight microsatellite markers were utilized to examine the population structure of *Labeo dero*, based on the genetic differentiation discovered by these markers. Five distinct genetic stocks of *Labeo dero* were found across its natural distribution. The mean *Fst* value over all collections and microsatellite loci in the *Labeo dero* wild population in several rivers was determined to be 0.019, meaning that genetic differentiation accounts for 1.9% of the overall genetic variability (Chaturvedi *et al*., 2011). Six loci were effectively crossed amplified using seventy *Sperata aor* samples each from four places along the Ganga River. Across all studied populations, the mean observed and anticipated heterozygosities were 0.971 and 0.913, respectively using microsatellite markers. High levels of genetic variety and the absence of inbreeding indicate that *S. aor* populations in the Ganga are stable at the moment (Nazir and Khan 2017).

Analysis of Molecular Variance

The division of molecular variability at several arbitrarily specified hierarchical levels from the individual level to the population level was the basis for a nested analysis of variance in this statistical method (Fig. 3). To examine the overall genetic heterogeneity of the samples, the analysis of molecular variance (AMOVA) framework (Excoffier *et al*., 1992) was applied in the program ARLEQUIN Version 3.5 (Excoffier and Lischer 2010). Within each of the five populations of snow trout in Kumaun and Garhwal Himalayan regions, the hierarchical analysis of molecular variation (AMOVA) demonstrated a considerable divide between the populations in the entire sample (*Fst* = 0.65411 for *ATPase 6/8* and *Fst* = 0.55573 for *COI*). Among *S. aor* populations, the AMOVA test revealed a substantial genetic difference. Within individuals, a value of 94% was recorded, while stocks of *S. aor* across the Ganga River showed 6% of the variation (Nazir and Khan 2017). The majority of the variance was discovered to be within populations of *Mastambelus armatus* in Yamuna River, according to the AMOVA study. Further, *Fst* analysis showed a negative low pairwise *Fst* value (-0.001258) between Kalsi (Y1) and after the Asan Barrage (A3), indicating that these groups experienced significant genetic exchange events. This indicates that the areas were either connected at one time or experience frequent flooding, and that both of these processes allowed populations to mix (Thapliyal *et al*., 2020). There is significant genetic divergence across the populations of the Satluj, Gomti, Yamuna, Brahmaputra, and Mahanadi rivers, as indicated by the congruence of AMOVA results with significant pairwise and overall Fst values (Gupta *et al*., 2013). Taking into account the four groups of Satluj, Ganga, Mahanadadi, and Narmada, a three-way AMOVA was conducted. Differences between groups accounted for 19.01% of the overall genetic variance, according to the hierarchical analysis of molecular variance. 73.71% was ascribed to variation within populations of *Chitala chitala* (*Fst*=0.263, $p<0.05$; *Fct*=0.190, $p<0.05$), while 7.27% was ascribed to variation among populations within groups (*Fsc*=0.090, $p<0.05$**)** (Dutta *et al*., 2020). Sequence analysis of mitochondrial DNA from seven geographically diverse areas along the Indian coast was used to evaluate the population structure of yellow fin tuna. For 321 yellow fin samples, a 500 bp section of the D-loop region was sequenced and examined. Significant genetic differentiation was seen among the three groups (VE, AG), (KO, TU, VI, PB) assessed by hierarchical analysis of molecular variance (*ΦST*=0.03844, $P < 0.001$(Kunal *et al*., 2013).

Estimation of Haplotype and Nucleotide Diversity

When alleles in an organism are inherited jointly from a single parent, the resultant genotype is known as a haplotype (haploid genotype) (Barry *et al*., 2016). The degree to which a specific haplotype is unique within a given population is measured by its haplotype diversity. It is denoted by H (Nei and Tajima 1981). In molecular genetics, the term "nucleotide diversity" is used to quantify the level of polymorphism within a population. This statistic, represented by π, is the average number of nucleotide changes per site between any two DNA sequences randomly selected from the sample population. A measure of genetic variety is nucleotide diversity (Nei and Li, 1979). Mitochondrial *ATPase 6* and *8* genes were used to determine the genetic stock structure of *R. canadum*, which is spread throughout Indian seas. In this study, 842 bp sequence of the *ATPase 6/8* genes identified 15 haplotypes, with a mean low nucleotide diversity (π=0.001) and high haplotype diversity as h=0.785 (Joy *et al*., 2016). The mitochondrial gene and *cytochrome b* sequences from four populations of *C. catla* were sequenced and examined to determine the genetic structure of the populations. Among the four populations under investigation, the twelve distinct haplotypes were found. Data made it abundantly evident that lotic water (Narmada River) and lentic water bodies (Tighra reservoir) contain the highest values of polymorphisms, parsimony, and haplotype diversity in feral/wild populations. Since the partial *cyto b* is polymorphic, it may be used as a marker to identify genetic divergence across populations based on ecological environment (Garg and Mishra 2018). By analyzing 842 bp of the full mitochondrial DNA *ATPase 6/8* genes, the genetic stock structure of *P. argenteus*, which is dispersed along the Indian coast, was determined. Sequencing was done on 83 silver pomfret (*P. argenteus*) that were collected from 4 coastal areas in India (Gujarat, Kerala, Tamil Nadu, and West Bengal). Among the 83 individuals, 24 haplotypes were found with nucleotide diversity (0.0025) and haplotype diversity (0.87) (Divya *et al*., 2015). Using two mitochondrial genes, *ATPase6/8* and *Cytb (Cytochrome b*), genetic divergence in wild populations of *T. tor* was examined. Concatenated sequences (1963 bp) contained 23 haplotypes, while 140 sequences of the *Cytb* (1121 bp) and *ATPase6/8* (842 bp) genes indicated 12 and 7 haplotypes, respectively. Sequencing investigation of mitochondrial areas showed low nucleotide and moderate haplotype diversities, indicating balanced selection (Sah *et al*., 2020). The *cytochrome b (cyt b)* mitochondrial DNA (mtDNA) sequences are used in this work to examine the genetic diversity and population structure of *C. chanos* along the Indian coast. 38 haplotypes were found by sequencing a 1100-bp *cyt b* mtDNA segment; the haplotype diversity value was 0.835, and the nucleotide diversity value was 0.00400. This

suggests that there is not much genetic differentiation among the *C. chanos* populations along the Indian coast (Jose *et al*., 2022). 18 haplotypes (h) with haplotype diversity of 0.9081±0.023 and nucleotide diversity of 0.0658 were found after the 668 bp *mtcox1* gene in *Puntius ticto* was sequenced. *Puntius* revealed striking similarities to *Puntius sarana*. Consequently, at the DNA sequence level, this marker demonstrated favorable effects for species identification, gene flow estimation, and genetic polymorphism (Garg *et al*., 2017). However, the summary of the various studies carried out by many scientist has been given in table-1 which showing study site wise and species specific molecular diversity among the fish species.

Phylogenetic Analysis

The study of the links and evolutionary histories between or among groups of species is known as phylogenetics. The methods of phylogenetic inference, which center on documented heritable features such as DNA sequences, protein, amino acid sequences, or morphology, are used to infer these relationships. Since all organisms are products of evolutionary processes, comprehending and expressing an organism in biological terms requires knowledge of its evolutionary past. In order to establish the evolutionary history of something. Three different kinds of information are required. The first is phenotypic, or the knowledge derived from expressed characteristics such as proteins, biochemical markers, and both internal and external morphology. The second type of information is genotypic, meaning it is derived from the genetic material within the cell. Finally, by comparing the homologies found in DNA and proteins, we can learn more about the phylogeny of that organism, which can be visually depicted as a phylogenetic tree (Patwardhan *et al*., 2014).

The phylogenetic tree has been effectively reconstructed and taxonomic ambiguity has been resolved by the application of mitochondrial genome molecular phylogeny investigations (Fig. 2). Using the Ion Torrent next-generation sequencing platform, the entire mitochondrial genome of Tor tor has been sequenced, covering over 1000 ×. ND1 gene divergence value is higher than *COI* gene in a comparative mitogenome analysis. It also reveals the existence of a unique genetic lineage within T. tor. The *Neolissochilus hexagonolepis* ((*T. sinensis* (*T. putitora, T. tor*), (*T. khudree, T. tambroides*)) is the phylogenetic relationship among the Mahseer group (Kumar *et al*., 2016). The outcomes further validated concatenated gene sequences' potential for better resolution. The median-joining network of the concatenated data set was used to perform the phylogeographic conclusions. The three most prevalent haplotypes

among the 61 total were H3, H10, and H12. Haplotypes H10 (in populations, except Chauka, Ken, Mahanadi, and Narmada) and H12 (represented by 11 populations, except Satluj, Sharda, and Mahanadi) were shared by all populations, with the exception of Gomti and tons. Four natural genetic stocks were found to exist, and a high degree of genetic differentiation was revealed by this investigation. Based on demographic factors, it is necessary to evaluate fine-scale organization using multilocus markers, especially in Gangetic Rivers. Mahanadi showed no evidence of an alternative haplotype, although Gomti and Brahmputra showed reduced diversities (Dutta *et al*., 2020). After RAPD markers were screened in three *Systoma sarana* populations, the results showed 0.05926 genetic differentiation (*Gst*), estimated gene flow between the populations was 0.3437, intra-population heterozygosity was 0.1518, and total heterozygosity was 0.3726. These results indicated high genetic polymorphism using RAPD markers, which means that allele frequencies and molecular polymorphism can be examined using RAPD markers. The RAPD approach is reliable and uncomplicated, and manageable (Garg and Mishra 2023).

Conclusions

A species' ability to adapt to changing environmental conditions is enhanced by genetic variety, which is essential to the species' survival. The use of DNA markers in population genetics is becoming widely accepted. Applications for genetic diversity data in evolution study, natural resource conservation and management, genetic enhancement initiatives, etc., are numerous. Theoretically, genetic variation throughout the genome can be observed and utilized with DNA markers. There are several uses for mitochondrial DNA. For the purpose of developing effective conservation strategies for the management of fisheries and aquaculture, molecular markers analysis gives vital information. The varied riverine system of India includes lakes, ponds, reservoirs, tributaries, streams, canals, and other features that are all home to a large number of fish.In this review river systems in India such as Godavari, Gomti, Brahmaputra, Satluj, Yamuna, Mahi, Mahanadi, Narmada, Krishna, Cauvery, Ganga, and Hooghly rivers, as well as their minor tributaries, have been documented that have significant Ichthyofaunal genetic diversity by using some molecular markers, Microsatellite markers, Allozymes, Mitochondrial genes(COX1, Cyto b, ATPase6/8) and RAPD. Additionally, these molecular markers shows promising results for analyzing the divergence as well as structure of populations at the population level. Hence, research in this field offers a lot of potential advantages. Some of the

most significant ones are the discovery of new evolutionary insights, the establishment of genetic enhancement initiatives, and the creation of conservation plans to preserve species' native genetic resources.

ACKNOWLEDGMENTS

The Authors with thanks of Honorable Vice Chancellor, Barkatullah University, Bhopal for their help and encouragements during the survey of research work. The Authors are also with thanking to laboratory colleagues and students for their support.

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Table 1: Data showing *Hd* and π values of different population of fishes in India

Populations	Haplotype diversity (Hd)	Nucleotide diversity (π)	Comments
Berach	0.6691±0.0591	0.00073±0.00062	Mitochondrial *cytob* gene was used to study genetic divergence in the natural population of *Tor tor*
Narmada-Tawa	0.4485±0.0858	0.00071±0.00058	
Narmada-Khargone	0.4819±0.1106	0.00073±0.00061	
Penganga	0.4248±0.0993	0.00114±0.00084	
Godavari	0.0000±0.0000	0.00000±0.00000	Revealed the genetic diversity in wild population of *Notopterus notopterus* by using mtDNA *ATPase 6/8* regions from five Indian rivers
Satluj	0.581±0.093	0.000836±0.000729	
Gomti	0.750±0.107	0.01890±0.001326	
Yamuna	0.766±0.070	0.001820±0.001275	
Brahmputra	0.773±0.083	0.002105±0.001474	
Mahanadi	0.618±0.054	0.001705±0.001194	
Cochin	0.6000	0.0028	Genetic stock structure of *Priacanthus hamrur* was ascertained with mitochondrial *cyt b* gene from Indian coastal waters
Mumbai	0.8000	0.0056	
Veraval	0.8000	0.0028	
Chennai	0.8000	0.0042	
Visakhapatnam	0.7000	0.0052	
Mahanadi	0.63235	0.00195	Revealed the genetic variation and population structure of *Cirrhinus mrigala* by mitochondrial
Godavari	0.50769	0.00168	
Krishna	0.54943	0.00212	

Kaveri	0.25057	0.00064	*ATPase 6* gene from peninsular rivers of India
Narmada	0.52644	0.00164	
Mahi	0.32468	0.00117	
Calicut	0.7091	0.001860	Estimated the genetic diversity and demographic history of *Chanos chanos* by using *cyt b* from Indian waters
Mandapam	0.9421	0.005173	
Cochin	0.8842	0.005789	
Tuticorin	0.7333	0.002888	
Chilika	0.8497	0.002810	

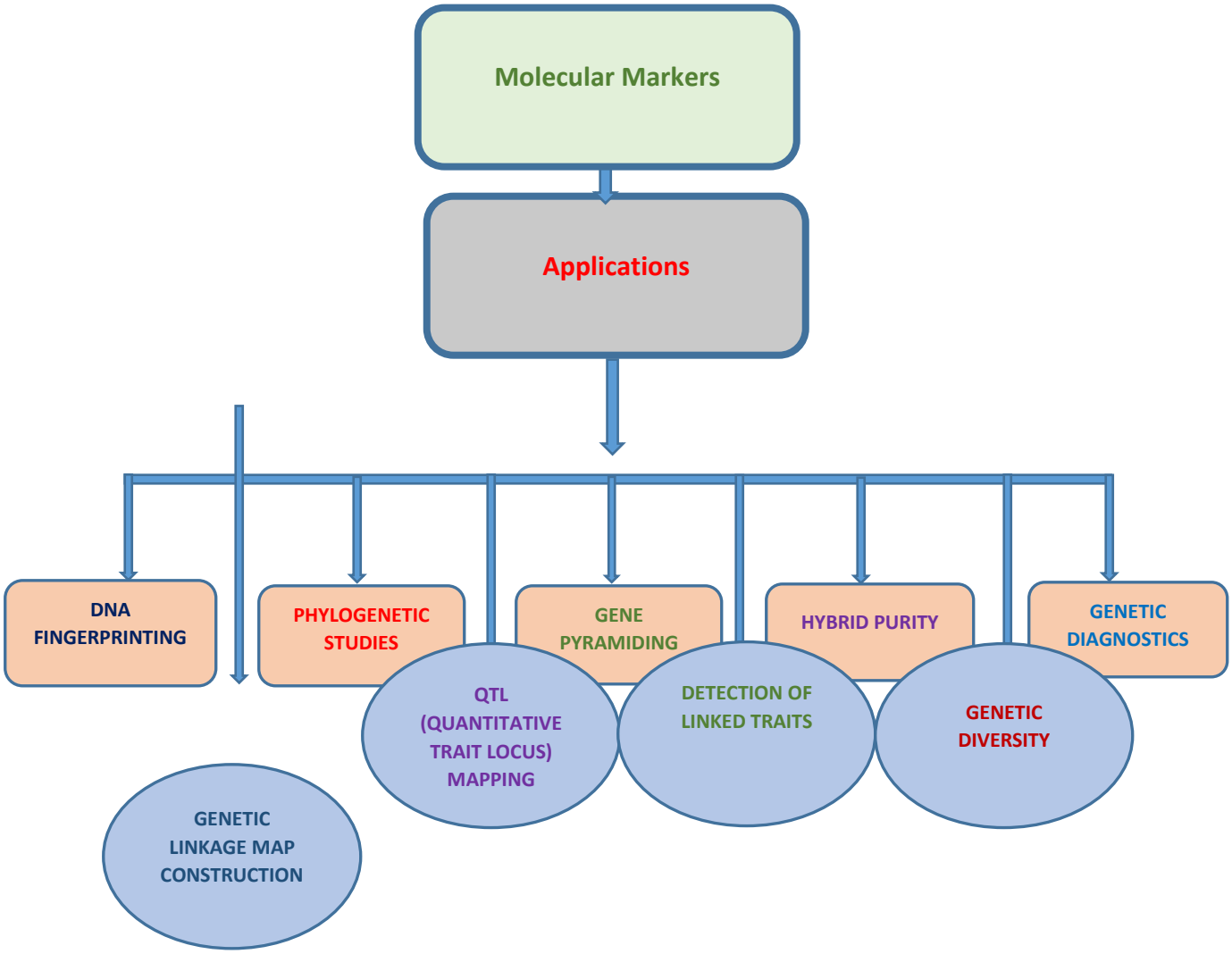

Fig. 1: Application of PCR based molecular markers for genetic studies

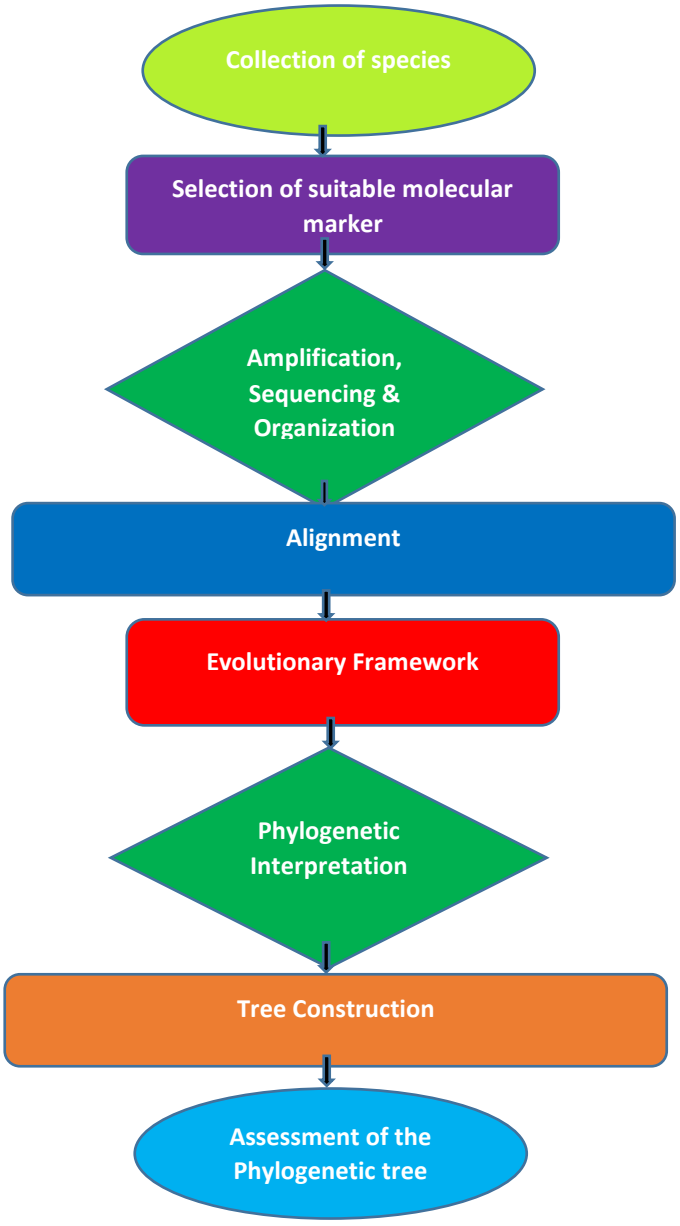

Fig. 2: Basic procedures for examining molecular phylogeny

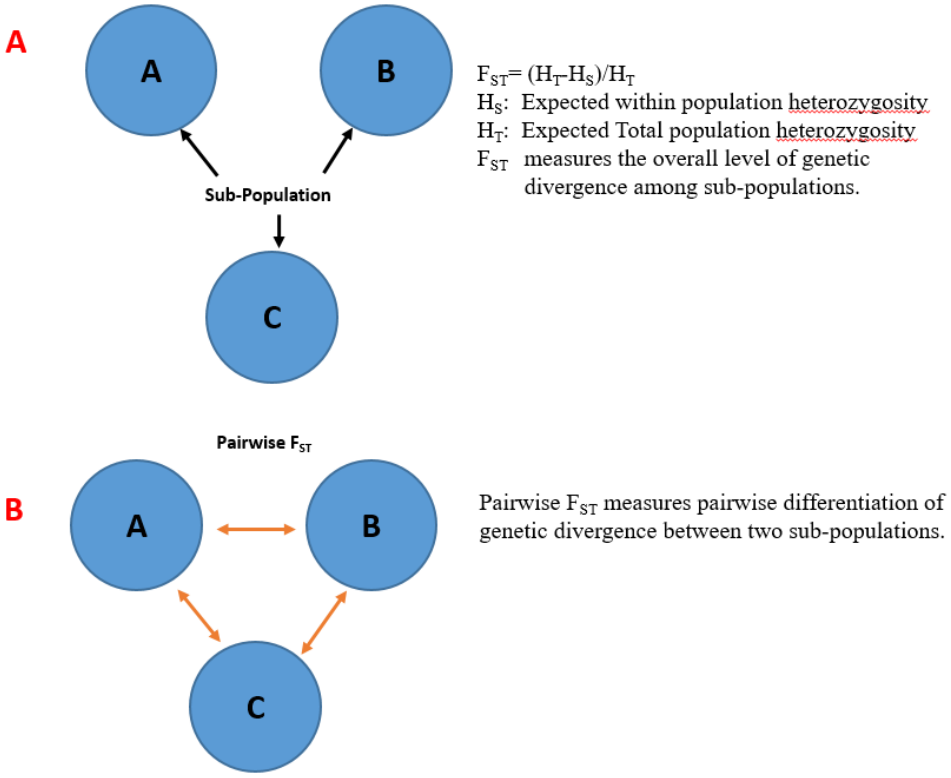

Fig. 3. Level of genetic divergene within and among populations.