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# Population Study of African Catfish *Clarias gariepinus* in India using Microsatellite DNA

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**Genetic variation between six populations of *Clarias gariepinus* were analyzed using six microsatellite loci. All the loci were highly polymorphic among all samples. The mean number of alleles and effective alleles per locus ranged from 4.667 to 5.833 and 2.000 to 6.000 respectively. The heterozyosities observed were found to be 0.739±0.012. Conformity to Hardy Weinberg Equilibrium using chi-square test showed that at 26 test out of 36 test (P<0.005) showed a significant value. The population differentiation ($F_{ST}$= -0.0170) was lower than gene flow (Nm= 0.8885) indicating heterozygosities in the population. The polymorphism information content (PIC) were greater than 0.6 in most of the loci indicating informative marker for the selected loci. The Nei's genetic distance was high between Pop1 and Pop6 with 5.669. The observations showed that due to allelic diversity and estimates of heterozygosity, these markers can be useful in the genus Clarias for population level analysis in the Indian Clariidae fishes using microsatellite DNA markers.**

**Key words**: Genetic variability, Polymorphism, Genetic distance, Neutrality, AMOVA.

The genus Clarias is the most common and popular of the family Clariidae(Teugels, 1986). *Clarias gariepinus* (Burchell, 1822) is of great economic importance as it is the most cultured catfish species in many countries(Garibaldi, 1996). African catfish *C. gariepinus* was introduced to India through trade channels (Thakur, 1996). Out of 15 genera of the family Clariidae, the genus Clarias alone has 32 African and 9 Asian valid species (Teugels and Adriaens, 2003). African catfish are known as air breathing fishes and they are hardy in nature, rich in nutrients and ability to withstand poorly oxygenated water which makes these fishes important for cultivation.

Measuring genetic diversity in fish stocks is essential for understanding the population structure and for effective management of these stocks (Okumusand Ciftci, 2003). The relationship between genes and phenotypic expression is different and significantly interacted by environmental variables. Thus, the population genetics is mainly focused on the mendelian traits in species for the identification of the specimen. These methods had limited application. Later in twentieth century to identify and characterize the genes molecular genetics was discovered the study of structure, function and dynamics of genes at molecular level has showed more application for the conservation of the species.

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A microsatellite is a simple DNA repeated sequence. Microsatellite occur approximately once every 10kbp, while minisatellite loci occur once every 1500 kbp in fish species (Wright, 1993). Microsatellite loci are distributed throughout vertebrate genomes (Waldbieser *et al.,* 2001).Due to high polymorphism microsatellite loci may be used as markers in studies of parentage, quantitative genetics and population genetics (Tautz, 1989). Microsatellites are useful for Genome mapping studies (O'Connell and Wright, 1997).

The aim of the present work was to evaluate genetic diversity and differentiation of *C. gariepinus* from selected place in India using microsatellite DNA markers from *C. gariepinus*.

## MATERIALS AND METHODS

### Sample Collection and DNA Extraction

A sample of 15specimens of *Clarias gariepinus* were collected from Vellore- Pop1, Chennai- Pop2 (Tamil Nadu); Bellandur Lake and Varthur Lake Bangalore- Pop3 (Karnataka); Thrissur- Pop4 (Kerala); Lucknow- Pop5 (UP) and Surat- Pop6 (Gujarat) as live fish (Figure1). The tissue sample (fin clips)for DNA extraction were cut and stored in sterile eppendorf tubes containing 95% ethanol.

Genomic DNA was isolated from fin tissue samples using the protease K digestion and phenol–chloroform extraction method (Sambrook *et al.,* 1989). Purified DNA was quantified and stored at -20$^{o}$*C*. The quality of DNA was tested by electrophoresis on 1% Agarose gel and the quantity was determined by using a spectrophotometer (Eppondrof).

### Amplification of Microsatellite Loci

Six primer pairs were used for PCR amplification in this study. Their details are tabulated in Table 1 (Galbusera *et al.,* 1996).The microsatellite DNA regions were amplified through PCR. The reaction was carried out in a 10 µl reaction volume containing 50 ng of template DNA, 0.25 µM of each primer, 0.25 mM of each dNTP, 1 unit of *Taq* DNA polymerase (GENEI Pvt. Ltd., Bangalore, India), and 1 µl reaction buffer containing 1.5 mM $MgCl_{2.}$

An oil-free thermal cycler (Master Cycler Gradient, Eppendorf, Germany) was used for conducting the polymerase chain reaction. The PCR condition for each marker was optimized and the annealing temperature for each marker was adjusted to yield clear bands. The Electrophoresis was conducted on 2% Agarose gel and scored by comparison to 100 bp standard DNA ladder (GENEI Pvt. Ltd., Bangalore, India), and the bands were analyzed using DNA Alpha view software 3.3.1.0 (Cell Biosciences).

### Statistical Analysis of Microsatellite Data

Genotype of each individual fish was determined and recorded from the gels for each microsatellite loci. Size of the bands representing particular alleles at the microsatellite loci were estimated using the DNA Alpha view software 3.3.1.0 (Cell Biosciences), number of alleles (Na), effective number of alleles (Ne), deviation from Hardy-Weinberg equilibrium (HWE), Analysis of Molecular Variance (AMOVA), F–Statistics ($F_{ST}$, $F_{IT}$, $F_{IS}$) population differentiation ($F_{ST}$) values and gene flow, assignment test, principal coordinate analysis (PCoA) were performed using the software GenAIEx 6.5(Peakall and Smouse, 2012). Polymorphic information content (PIC) using Microsatellite tool kit(Park, 2001). Linkage disequilibrium (LD)and private alleles were calculated using GENEPOP v.4.2 (Raymond and Rousset, 1995). A Bonferroni approach(Rice, 1989) was used to correct multiple simultaneous tests.The neutrality test and observed and expected value of homozygosity and heterozygosity was measured with software program POPGENE V 1.32 (Yeh,1997). A dendrogram was drawn based on the Nei's unbiased genetic distance (Nei, 1978) between the populations following Unweighted Pair Group method of Averages (UPGMA) using the software POPGENE V 1.32 (Yeh, 1997).The genetic structure and the degree of admixture of six catfish populations were investigated using the Bayesian clustering procedure of STRUCTURE v2.3(Pritchard, 2000) to identify the most probable groups (K) the best fit the data, we used the STRUCTURE HARVESTER(Earl and Von, 2012), which implements the Evanno method (Evanno *et al,*. 2005).

## RESULTS AND DISCUSSIONS

Microsatellites are co-dominant in nature and inherited in Mendelian fashion, revealing

polymorphic amplification products helping in characterization of individuals in a population (Chauhan and Kumar, 2010). A microsatellite marker is effective in detecting low level of genetic change like inbreeding, bottleneck effect, mutation, environmental pollution factors(Chistiakov *et al.,* 2006; Liu and Cordes, 2004). The development of new species – specific microsatellite primers is expensive and time consuming and the alternative is cross species amplification option is cheap and fast. Primers developed by this method have been successfully tested for cross species amplification on its related species in several fish species. Islam *et al*.(2008). In the present study we have designed six primers from *C. gariepinus* and evaluated for species amplification of microsatellite loci in *C. gariepinus*. All the six loci were amplified successfully and resulted in clearly scorable bands on the standard Agarose gel and visualized under Gel analyzer. All the 6 amplified microsatellite loci were polymorphic (100%) in all the populations. The allele frequency for all the six population is shown in the Figure 2.

**Molecular Diversity**

The Na ranged from 2 (Pop2-Cga09) to 8 (Pop1-Cga10) and Ne from 2.000 to 6.000 with a grand mean of 5.333±0.218 and 4.105±0.174 respectively. Lal *et al*.(2003) made a comparative study with *C. gariepinus* collected from India and Thailand, the Indian species had highest mean alleles per locus (1.688) while Thailand species mean alleles were slightly lower (1.375). Five microsatellite loci were analyzed in the study of Jafari et al(2014) were alleles Na ranged from 7- 20 and average Ne per locus were 14.4 and 14.1. The high Shannons index (I)was observed in Cga03 of Pop5 with 1.840 and lowest in Cga09 of Pop2 with 0.693. The grand mean of I was 1.486±0.044 showing high gene diversity in all population over all loci. The grand mean of fixation indices were -0.313±0.027 indicating an excess of heterozygous and outbreeding (Table 2). The allelic pattern for all population is shown in the Figure 3.

**Genetic Diversity**

Heterozygosity is an important evolutionary indicator in determining the dynamics and survival of populations (2009). Information obtained from microsatellite markers showed high genetic diversity among individuals and low diversity within individual. However, high observed heterozygosity was indicative of intra-population genetic structuring of *C.gariepinus* populations. All the loci Obs_Hom was less than Obs_Het ranging from 0.000 to 0.055 and 0.9444 to 1.000 respectively indicating outbreeding in the population due to decreased homozygosity. While Obs_Het was greater than Exp_Het at all loci Table2. In Agbebi *et al*. (2013) the mean observed heterozygosity (Het_Obs) over loci and populations is 0.450±0.050 and the mean expected heterozygosity (Het_exp) over loci and populations is 0.896±0.011. In the Nazia & Azizah (2014) study the *C. macrocephalus*, the observed and expected heterozyosities varied from 0.33 to 0.967 with an average of 0.696 and from 0.33 to 0.942 with an average of 0.789 respectively. The observed and

**Table 1.** Showing the details of six microsatellite primer (Galbusera *et a*l, 1996)

| S.No | Specimen | Accession No. | Locus | Primer 5'-3' |
|---|---|---|---|---|
| 1 | *Clarias gariepinus* | U30862 | *Cga01* | F-GGCTAAAAGAACCCTGTCTG<br>R-TACAGCGTCGATAAGCCAGG |
| 2 | *Clarias gariepinus* | U30863 | *Cga02* | F-GCTAGTGTGAACGCAAGGC<br>R-ACCTCTGAGATAAAACACAGC |
| 3 | *Clarias gariepinus* | U30864 | *Cga03* | F-CACTTCTTACATTTGTGCCC<br>R-ACCTGTATTGATTTCTTGCC |
| 4 | *Clarias gariepinus* | U30867 | *Cga06* | F-CAGCTCGTGTTTAATTTGGC<br>R-TTGTACGAGAACCGTGCCAGG |
| 5 | *Clarias gariepinus* | U30871 | *Cga09* | F-CGTCCACTTCCCCTAGAGCG<br>R-CCAGCTGCATTACCATACATGG |
| 6 | *Clarias gariepinus* | U30870 | *Cga10* | F-GCTGTAGCAAAAATGCAGATGC<br>R-TCTCCAGAGATCTAGGCTGTCC |

expected heterozygosities in *Leptobotia elongata* ranged from 0.375 to 1.000 and from 0.467 to 0.957, respectively (Dongqi *et al.*,2014).

**Polymorphic information content (PIC)**

In the study of Dashab et al.(Dashab*et al.*, 2011) the PIC per locus ranged from 0.45 to 0.76, while in our present study the PIC ranged from 0.3 to 0.8. Markers with PIC values >0.4 are considered moderately informative and those with values >0.7 are considered highly informative (Hildebrand *et al.*, 1992). The highest was detected in Cga03 of Pop6 (0.81) and lowest in Cga09 of Pop2 (0.37). except one all the loci at all the population exhibited PIC>0.5 indicating an informative marker for this study. These parameters indicate that the six populations belonged to the level of high polymorphism and genetic variations were also high.

**Private Alleles**

The homologous markers in *C. gariepinus* showed high Nm with 0.2375 and less number of private alleles (0.1860).

**Hardy-Weinberg expectations**

Hardy-Weinberg equilibrium is based on the random mating in a population; deviations from HWE in wild populations are expected (Dixon *et al.*, 2008). The probability test provided the evidence that the observed allele frequencies in

**Table 2.** Statistics of allelic diversity of six populations of *C. gariepinus* for six microsatellite loci

| Pop | Locus | N | Na | Ne | I | Ho | He | uHe | F | P |
|---|---|---|---|---|---|---|---|---|---|---|
| Pop1 | *Cga01* | 15 | 5.000 | 3.191 | 1.326 | 1.000 | 0.687 | 0.710 | -0.456 | 0.000 |
| | *Cga02* | 15 | 7.000 | 4.945 | 1.748 | 1.000 | 0.798 | 0.825 | -0.253 | 0.000 |
| | *Cga03* | 15 | 5.000 | 3.020 | 1.316 | 1.000 | 0.669 | 0.692 | -0.495 | 0.132 |
| | *Cga06* | 15 | 6.000 | 5.488 | 1.745 | 1.000 | 0.818 | 0.846 | -0.223 | 0.000 |
| | *Cga09* | 15 | 7.000 | 4.500 | 1.684 | 1.000 | 0.778 | 0.805 | -0.286 | 0.724 |
| | *Cga10* | 15 | 8.000 | 5.233 | 1.853 | 1.000 | 0.809 | 0.837 | -0.236 | 0.042 |
| Pop2 | *Cga01* | 15 | 4.000 | 2.761 | 1.140 | 0.733 | 0.638 | 0.660 | -0.150 | 0.713 |
| | *Cga02* | 15 | 4.000 | 2.922 | 1.215 | 0.800 | 0.658 | 0.680 | -0.216 | 0.098 |
| | *Cga03* | 15 | 5.000 | 2.632 | 1.235 | 0.867 | 0.620 | 0.641 | -0.398 | 0.554 |
| | *Cga06* | 15 | 5.000 | 3.846 | 1.462 | 0.867 | 0.740 | 0.766 | -0.171 | 0.000 |
| | *Cga09* | 15 | 2.000 | 2.000 | 0.693 | 1.000 | 0.500 | 0.517 | -1.000 | 0.000 |
| | *Cga10* | 15 | 6.000 | 3.913 | 1.539 | 1.000 | 0.744 | 0.770 | -0.343 | 0.000 |
| Pop3 | *Cga01* | 15 | 7.000 | 5.000 | 1.766 | 0.933 | 0.800 | 0.828 | -0.167 | 0.066 |
| | *Cga02* | 15 | 6.000 | 4.128 | 1.568 | 1.000 | 0.758 | 0.784 | -0.320 | 0.000 |
| | *Cga03* | 15 | 5.000 | 3.846 | 1.462 | 0.933 | 0.740 | 0.766 | -0.261 | 0.046 |
| | *Cga06* | 15 | 6.000 | 3.629 | 1.482 | 0.867 | 0.724 | 0.749 | -0.196 | 0.000 |
| | *Cga09* | 15 | 5.000 | 4.128 | 1.493 | 1.000 | 0.758 | 0.784 | -0.320 | 0.000 |
| | *Cga10* | 15 | 4.000 | 2.922 | 1.182 | 0.667 | 0.658 | 0.680 | -0.014 | 0.244 |
| Pop4 | *Cga01* | 15 | 4.000 | 3.782 | 1.357 | 1.000 | 0.736 | 0.761 | -0.360 | 0.000 |
| | *Cga02* | 15 | 3.000 | 2.830 | 1.068 | 1.000 | 0.647 | 0.669 | -0.546 | 0.021 |
| | *Cga03* | 15 | 7.000 | 5.556 | 1.804 | 1.000 | 0.820 | 0.848 | -0.220 | 0.000 |
| | *Cga06* | 15 | 4.000 | 2.941 | 1.194 | 1.000 | 0.660 | 0.683 | -0.515 | 0.000 |
| | *Cga09* | 15 | 6.000 | 4.639 | 1.642 | 1.000 | 0.784 | 0.811 | -0.275 | 0.000 |
| | *Cga10* | 15 | 6.000 | 4.455 | 1.621 | 1.000 | 0.776 | 0.802 | -0.289 | 0.000 |
| Pop5 | *Cga01* | 15 | 4.000 | 3.285 | 1.273 | 1.000 | 0.696 | 0.720 | -0.438 | 0.000 |
| | *Cga02* | 15 | 4.000 | 3.237 | 1.262 | 1.000 | 0.691 | 0.715 | -0.447 | 0.000 |
| | *Cga03* | 15 | 7.000 | 5.769 | 1.840 | 1.000 | 0.827 | 0.855 | -0.210 | 0.000 |
| | *Cga06* | 15 | 6.000 | 4.839 | 1.663 | 1.000 | 0.793 | 0.821 | -0.261 | 0.000 |
| | *Cga09* | 15 | 6.000 | 5.844 | 1.778 | 1.000 | 0.829 | 0.857 | -0.206 | 0.000 |
| | *Cga10* | 15 | 4.000 | 3.913 | 1.375 | 1.000 | 0.744 | 0.770 | -0.343 | 0.000 |
| Pop6 | *Cga01* | 15 | 4.000 | 3.600 | 1.330 | 1.000 | 0.722 | 0.747 | -0.385 | 0.000 |
| | *Cga02* | 15 | 6.000 | 4.839 | 1.663 | 1.000 | 0.793 | 0.821 | -0.261 | 0.000 |
| | *Cga03* | 15 | 6.000 | 6.000 | 1.792 | 1.000 | 0.833 | 0.862 | -0.200 | 0.000 |
| | *Cga06* | 15 | 6.000 | 4.018 | 1.521 | 1.000 | 0.751 | 0.777 | -0.331 | 0.000 |
| | *Cga09* | 15 | 6.000 | 5.056 | 1.703 | 1.000 | 0.802 | 0.830 | -0.247 | 0.000 |
| | *Cga10* | 15 | 6.000 | 5.056 | 1.684 | 1.000 | 0.802 | 0.830 | -0.247 | 0.000 |

N- Sample Size, No. Alleles, Na- No. Effective Alleles, I- Information Index, Ho- Observed He- Heterozygosity, uHe- Expected and Unbiased Expected Heterozygosity, F- Fixation Index and P- HWE $P < 0.05$.

**Table 3.** The overall Ewens-Watterson test for neutrality

| Locus | Obs. F | Min F | Max F | Mean* | SE* | L95* | U95* |
|---|---|---|---|---|---|---|---|
| *Cga01* | 0.0545 | 0.0417 | 0.7771 | 0.1220 | 0.0014 | 0.0759 | 0.2250 |
| *Cga02* | 0.0651 | 0.0385 | 0.7608 | 0.1110 | 0.0011 | 0.0697 | 0.1970 |
| *Cga03* | 0.0523 | 0.0357 | 0.7450 | 0.1020 | 0.0009 | 0.0654 | 0.1822 |
| *Cga06* | 0.0516 | 0.0370 | 0.7528 | 0.1067 | 0.0012 | 0.0677 | 0.1945 |
| *Cga09* | 0.0490 | 0.0357 | 0.7450 | 0.1001 | 0.0008 | 0.0651 | 0.1696 |
| *Cga10* | 0.0461 | 0.0333 | 0.7297 | 0.0932 | 0.0006 | 0.0597 | 0.1554 |

* These statistics were calculated using 1000 simulated samples.

**Table 4.** Pairwise population $F_{ST}$ values

| Pop1 | Pop2 | Pop3 | Pop4 | Pop5 | Pop6 | |
|---|---|---|---|---|---|---|
| 0.000 | | | | | | Pop1 |
| 0.172 | 0.000 | | | | | Pop2 |
| 0.122 | 0.178 | 0.000 | | | | Pop3 |
| 0.134 | 0.175 | 0.144 | 0.000 | | | Pop4 |
| 0.132 | 0.168 | 0.139 | 0.142 | 0.000 | | Pop5 |
| 0.129 | 0.164 | 0.133 | 0.131 | *0.113* | 0.000 | Pop6 |

Pop1-Vellore; Pop2-Chennai; Pop3-Bangalore; Pop4-Thrissur; Pop5-Lucknow; Pop6-Surat.

**Table 5.** F-Statistics and Gene Flow (Nei, 1987)

| Locus | $F_{IS}$ | $F_{ST}$ | $F_{IT}$ | Nm* |
|---|---|---|---|---|
| Cga01 | -0.3247 | 0.2459 | 0.0011 | 0.7665 |
| Cga02 | -0.3350 | 0.2255 | -0.0340 | 0.8587 |
| Cga03 | -0.2863 | 0.2070 | -0.0201 | 0.9577 |
| Cga06 | -0.2779 | 0.2115 | -0.0076 | 0.9318 |
| Cga09 | -0.3480 | 0.2199 | -0.0515 | 0.8868 |
| Cga10 | -0.2500 | 0.2079 | 0.0099 | 0.9524 |
| All | -0.3031 | 0.2196 | -0.0170 | 0.8885 |

* Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$.

most of the loci significantly deviated ($P < 0.05$) from that expected under hardy Weinberg equilibrium in all six populations after sequential Bonferroni correction was made to the probability levels, 26 test out of 36 test were significant. Except Pop4 and Pop2 at locus Cga02, at locus Cga09 only Pop1 displayed NS, at locus Cga10 Pop1 and Pop3, at locus Cga01 Pop3 and Pop2, and at locus Cga03 Pop2 and Pop3 as shown in the Table 2. In a study by Nazia & Azizah (2014) at the locus NCm-F8 it significantly deviated from HWE ($P<0.05$). All sites in Prochilodus magdalenae presented

**Table 6.** Genetic Distance and Identity (Nei, 1978)

| Pop ID | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | **** | 0.0220 | 0.1716 | 0.0791 | 0.0312 | 0.0035 |
| 2 | 3.8164 | **** | 0.0198 | 0.0477 | 0.0336 | 0.0092 |
| 3 | 1.7627 | 3.9218 | **** | 0.0520 | 0.0283 | 0.0223 |
| 4 | 2.5367 | 3.0436 | 2.9560 | **** | 0.0125 | 0.0518 |
| 5 | 3.4676 | 3.3926 | 3.5645 | 4.3807 | **** | 0.1357 |
| 6 | 5.6694 | 4.6901 | 3.8026 | 2.9606 | 1.9975 | **** |

Nei's genetic identity (above diagonal) and genetic distance (below diagonal). Pop1-Vellore; Pop2-Chennai; Pop3-Bangalore; Pop4-Thrissur; Pop5-Lucknow; Pop6-Surat

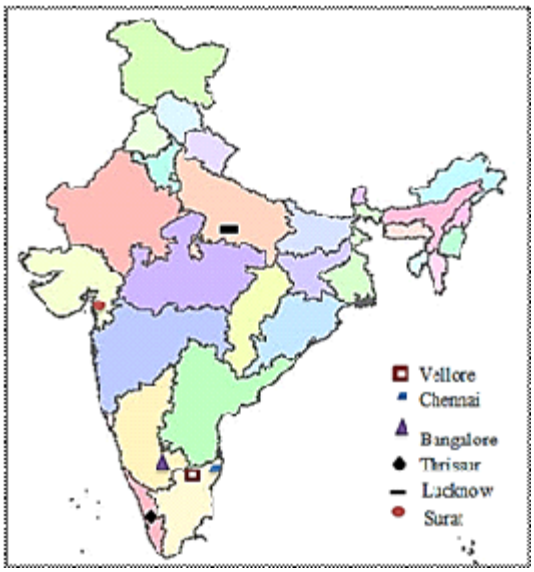
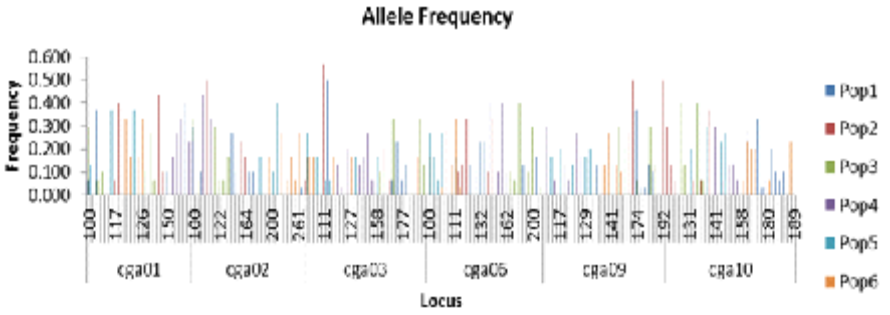



**Fig. 1.**Map of India showing the sample collection site

departures from the Hardy-Weinberg Expectations (HWE) for all loci (P<0.01) in the study of Gilberto and Juan (Gilberto and Juan, 2014).

**Analysis of Molecular Variance (AMOVA)**

To determine whether populations were genetically structured we used analysis of molecular variance (AMOVA). High genetic variation between and within populations indicate utilization of natural populations for either genetic improvement program or for aquaculture. In Baluchi sheep among population 2.4% and within population 97.6% variance was exhibited in the study of Dashab *et al*. whereas in our study 19% variance was observed among populations of *H. fossilis*. The stock obtained from the lakes to study the genetic diversity between populations, which suggests that farmers can use the local populations for aquaculture, because they are already adapted to that particular environment (Na-Nakorn *et al.*, 1999)(Figure 4).




Pop1- Vellore, Pop2- Chennai, Pop3- Bangalore, Pop4- Thrissur, Pop5- Lucknow, Pop6- Surat

**Fig 2.**Allele frequency of all the six populations

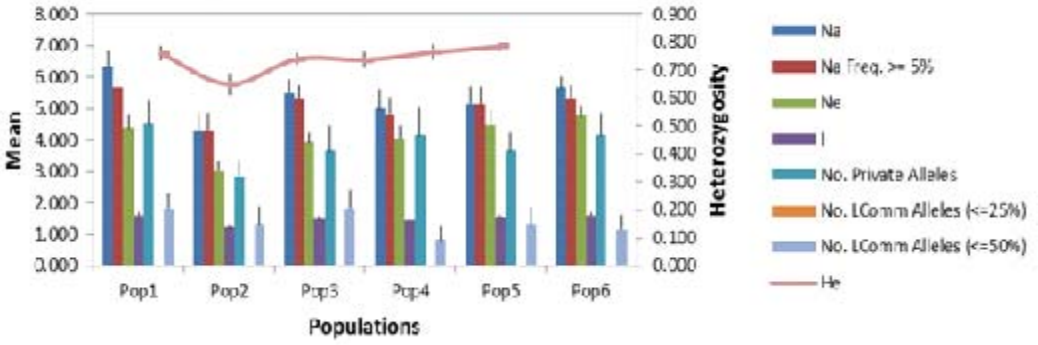



**Fig 3.**Allelic patterns across populations-Na- No. of different alleles, Na (Freq.>=5%)- No. of different alleles with a frequency>=5%, Ne-effective number of alleles, I- Shannon's Information Index, No. of private alleles- No. of alleles unique to a single population, No. LComm Alleles (<=25%) - No.of locally common alleles (freq.>=5%) found in 25% or fewer populations.

**Linkage Disequilibrium**

The observed haplotype frequencies were also used to study the allelic association for the eight loci. Using Fisher´s exact test to compare observed and expected haplotype frequencies we showed that the loci within each of the 13 linkage groups were in LD ($P < 0.003$), after adjustment for multiple testing), while linkage pair at Cga01 &Cga02 and Cga01 & Cga09 groups could not be shown to be in LD after adjustment for multiple testing among the six microsatellite loci at 95% confidence levels with Markov Chain Correction.

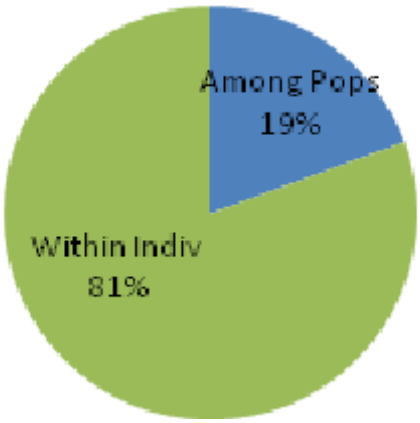



**Fig. 4.** Analysis of Molecular Variance

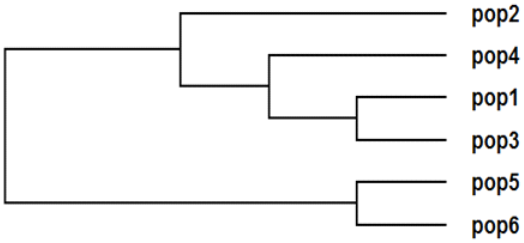



**Fig. 5.** Dendrogram Nei's (1978) UPGMA with 1000 Bootstrap values- Pop1-Vellore; Pop2-Chennai; Pop3-Bangalore; Pop4-Thrissur; Pop5-Lucknow; Pop6-Surat

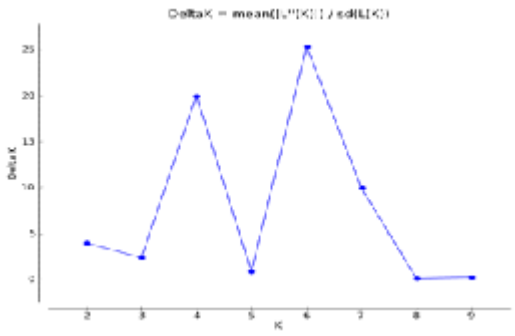


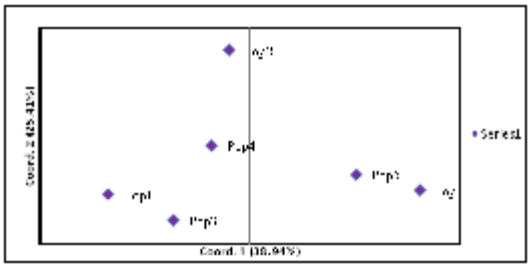
**Fig. 6.** Delta K value

**Neutrality Test**

The microsatellite markers have the specific property, as they are neutral to selection and the neutrality was tested by Ewens-Watterson test which compares the observed level of homozygosity (Obs F) to that which is expected under neutrality. None of the loci were neutral as all lied outside the lower and upper limit of 95% confidence region of expected F value as shown in the Table 3.

**Genetic Differentiation and Gene Flow**

Gametal correlation co-efficient ($F_{ST}$) also known as co-efficient of inbreeding and gene flow (Nm) were computed to estimate the differences between population. According to wright criteria (Wright, 1978) $F_{ST}$ value less than 0.05 indicate the low differentiation among communities; hence the results represent high between populations. The important F-Statistics parameters: $F_{IS}$, $F_{IT}$ and $F_{ST}$ were recorded higher at loci (-0.2500, 0.2459 and -0.0099) Cga10, Cga01 and Cga10 respectively (Table 4). Negative values of $F_{IS}$ indicates the presence of excess of heterozygotes in the population. In our study Fis at all loci exhibited negative values showing distinct relationship between the individuals. The Fis heterozygotes were high at the loci Cga02 (0.3350) and lowest at Cga10 (-0.2500). the high genetic differentiation was observed between Pop2 and Pop3 while lowest at Pop5 and Pop6. The $F_{ST}$ (population differentiation) shows highest value of 0.153 and lowest with 0.001 in *Cyprinus carpio* (Jewel *et al.*, 2006). Li *et al.* (2009) notes that when Nm>1 and Nm<1, then



Pop1-Vellore; Pop2-Chennai; Pop3-Bangalore; Pop4-Thrissur; Pop5-Lucknow; Pop6-Surat

**Fig. 7.** Principal Coordinate Analysis of population accessions based on eight microsatellite markers indicating separation or the breed relationship

genetic differentiation occurred due to number of migrant and gene migrant respectively. $F_{ST}$ was lower at all loci and all population compared to Nm, indicating more gene flow and heterozygosity in the selected loci. Highest Nm was seen in the locus Cga03 (0.9577) and lowest at Cga01 locus (0.7665). Highest Nm was exhibited between Pop5 and Pop6 (1.962) (Table 5).

**Genetic Distance and Similarity**

The genetic distance and identity was measured for six populations using the unbiased UPGMA method and tabulated as shown in the Table 6. The genetic identity between the Pop1 and Pop3 showed more similarity (0.1716) and less between Pop1 and Pop6 (0.0035). This is also shown in the dendrogram in UPGMA tree **(**Nei, 1978**)** (Figure 5).In the study of Agbebi *et al.* (2013) *Hetrobranchus bidorsalis* and *C. gariepinus* formed two different clusters with a bootstrap value of 72.

**Cluster Analysis**

*I*n the STRUCTURE analysis the value of ”K was high as K=6 dividing the individuals according to K value clusters. The output file of STRUCTURE HARVESTER is shown in Figure 6.In the study of Rueda *et al*. (Rueda *et al*., 2013)the STRUCTURE analysis indicated that the most probable number of clusters in the total sample was k=3 for Prochilodus lineatus in the lower Uruguay river.

The two-dimensional Principal Coordinate Analysis (PCoA) plot (Figure 7) shows that the first principal coordinate accounts for 38.94% of total variation and separates the Pop1, 2, 3 and 4 from Pop5 and 6 Populations. The second principal coordinate (25.41% of total variation) separated most individuals from those of Pop2 & 4 from Pop1 & 3; Pop5 & 6 was found in single cluster.

Assignment tests are essentially based on calculations of the likelihood of multilocus genotypes in population given the allele frequencies of the populations considered. An individual is assigned to the population for which it has the highest likelihood.

## CONCLUSIONS

From this study its concluded, that the genetic diversity of *Clarias gariepinus* is high for the selected loci among the six population. This is again proved by increased heterozygosity levels showing gene flow within their region. The analysis of Hardy Weinberg equilibrium exhibits evolutionary progress. The PIC describes the selected microsatellite DNA regions are informative for this study. Further analysis of cross species amplification can help in categorizing markers in and among Clariidae family.

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