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**Research Article**

# GENETIC DIVERSITY OF AFRICAN CATFISH *CLARIAS GARIEPINUS* IN SOUTH INDIA EVALUATED BY MICROSATELLITE DNA

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## ABSTRACT

The Clariid species, *Clarias gariepinus* is native to Africa and one of the most cultivated fish in the world. The aim of this research was to evaluate the genetic diversity of this fish from selected places of Karnataka and Tamil Nadu of south India, using *Clarias macrocephalus* microsatellite DNA markers. This cross amplification was done using two primers (*Cmac6 and Cmac11*). The mean number of alleles per locus ranged from 0.500 to 5.000. The heterozyosities observed were found to be 0.1625±0.0707, while 100% polymorphism was observed in *C. gariepinus*. Conformity to Hardy Weinberg Equilibrium using chi-square test showed that at P<0.001 all the loci showed a significant value. The genetic distance of Nei's (1978) indicating that the genetic distance between the four population forming two clusters one Poondi Lake and Sholavaram Lake of Chennai; and Bellandur Lake and Varthur Lake of Bangalore on the other cluster.

**Keywords**: Microsatellite, *Clarias gariepinus*, Genetic variability, Cross amplification, Genetic distance, Dendrogram, AMOVA.

## INTRODUCTION

The family Clariidae belongs to the Siluriformes present in Southeast Asia and Africa (Na-Nakorn *et al.*, 1999). *Clarias gariepinus* was introduced to India through trade channels (Mohindra *et al.*, 2007). This fish is a freshwater fish with fast growth rate, hardiness, efficient feed utilization, and the ability to survive in poorly oxygenated waters and widely tolerant of extreme environmental conditions thus it is a preferred fish for culture. As this is an air breathing catfish, this fish is sold as live fish in the market. These features make this species a potential candidate for aquaculture.

Genetic variation helps population to adapt to changes in its environment (Gjedrem, 2005). The variable populations will generally respond better than non-variable ones to environmental changes because more exploitable variations remain. Population genetics is a field of biology that studies the genetic composition of biological populations (allele frequency distributions) and the changes in genetic composition that result from evolutionary forces of which natural selection, genetic drift, mutation and gene flow are considered to be the major ones that influence gene frequencies in both natural and cultured populations (Hallerman, 2003).

Microsatellite markers are highly polymorphic markers used to identify the specific locations on the chromosome associated with the species. Microsatellite loci are distributed throughout vertebrate genomes (Waldbieser *et al.*, 2001). As the size of the locus is small it can be easily amplified by polymerase chain reaction (PCR). Due to high polymorphism microsatellite loci may be used as markers in studies of parentage, quantitative genetics and population genetics (Tautz, 1989).

The aim of the present work was to evaluate genetic diversity and differentiation of *C. gariepinus* from selected place of Karnataka and Tamil Nadu, South India using microsatellite DNA from *C. macrocephalus*.

## MATERIALS AND METHODS

### Sample Collection and DNA Extraction

A sample of 20 specimens of *Clarias gariepinus* were collected from Poondi Lake and Sholavaram Lake,

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Chennai, Tamil Nadu and Bellandur Lake and Varthur Lake Bangalore, Karnataka as live fish (Figure 1). The tissue sample (fin clips) for DNA extraction were 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**

Two primer pairs *Cmac6* and *Cmac*11 (Table 1) by (Sukkorntong *et al.,* 2008) were used for PCR amplification in this study. Their details are tabulated in Table 1.The microsatellite DNA regions were amplified through PCR. The reaction was carried out in a 10 µl reaction volume containing 50ng 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 50bp standard DNA ladder (GENEI Pvt. Ltd., Bangalore, India), and the bands were analyzed using DNA Alpha view software 3.3.1.0 (Cell Biosciences).

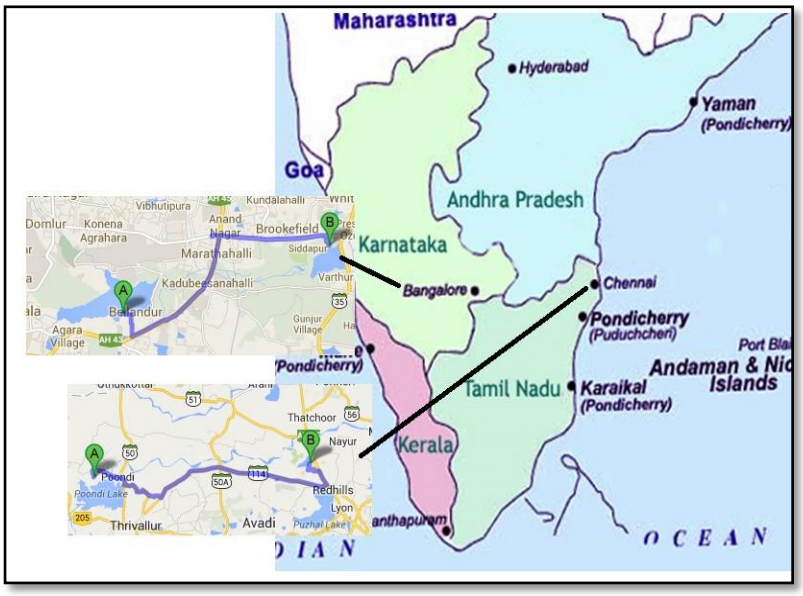


**Figure 1.** Map of India showing the sample collection site.

**Table 1.** Showing the details of two microsatellite primer.

| S. No. | Primer sequence (5'→3') | Locus | Annealing temperature (°C) | Clone accession No. | Reference |
|---|---|---|---|---|---|
| 1 | F-GCACAGTTGTCAAGGGCTTCTGC<br>R-TGTGTGTGGACATGTGGTACAGCC | Cmac11 | 56.6 | EU179753 | (Sukkorntong, *et al.*, 2008) |
| 2 | F- GCACGAGGGGGAGACTGACGA<br>R- TGGGCACAGGCATCAGGACT | Cmac6 | 56.6 | EU179743 | (Sukkorntong, *et al.*, 2008) |

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 50bp 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) were performed using the software GenAIEx 6.5 (Peakall and Smouse, 2012; Peakall and Smouse, 2006). Polymorphic information content (PIC) using Microsatellite tool kit (Park, 2001).The observed and expected value of homozygosity and heterozygosity, F–Statistics ($F_{ST}$, $F_{IT}$, $F_{IS}$) population differentiation ($F_{ST}$) values and gene flow. 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).

## RESULTS

Microsatellite markers developed for *C. macrocephalus*, cross amplified in *C.gariepinus*. This was used for

analyzing the population genetic structure of C. *gariepinus*. Both the loci (*Cmac11 and Cmac06*) were amplified successfully and resulted in clearly scorable bands on the standard Agarose gel and visualized under Gel analyzer.

### PCR amplification

Both the microsatellite loci were successfully amplified in all the four populations, fragments ranging from 153 to 215 bp (*Cmac*6) and 170-210bp (*Cmac11)* in length. The number of alleles per locus was 3 to 6, totally 28 alleles, 4.5 on an average as shown in the (Table 2). The allelic frequency and allelic patterns across populations is shown in the (Figure 2 and 3).

**Table 2.** Showing allelic variability between four populations.

| Pop | Locus | N | Size bp | Na | Ne |
|---|---|---|---|---|---|
| Poondi Lake | Cmac6 | 20 | 196-215 | 3.000 | 2.299 |
| | Cmac11 | 20 | 182-200 | 5.000 | 3.404 |
| Sholavaram Lake | Cmac6 | 20 | 153-175 | 4.000 | 2.254 |
| | Cmac11 | 20 | 185-192 | 5.000 | 4.211 |
| Bellandur Lake | Cmac6 | 20 | 190-210 | 6.000 | 4.520 |
| | Cmac11 | 20 | 172-210 | 4.000 | 2.402 |
| Varthur Lake | Cmac6 | 20 | 154-180 | 4.000 | 3.687 |
| | Cmac11 | 20 | 180-195 | 5.000 | 3.721 |

Locus name , number of sample (N), Size range in bp, Number of average alleles (Na), Number of effective alleles (Ne).

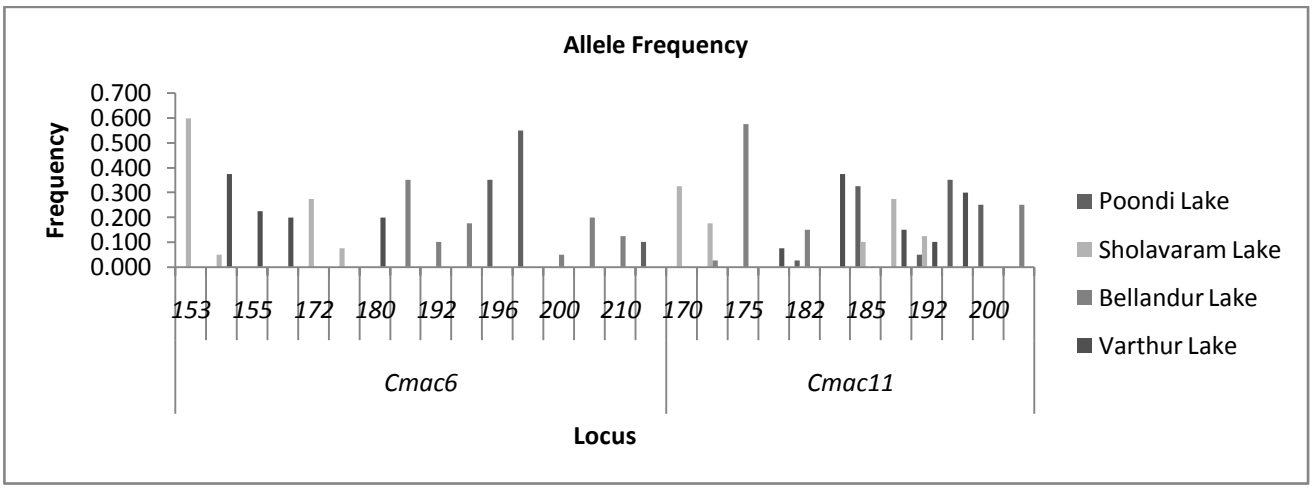


**Figure 2.** Allele frequency of all the four populations.

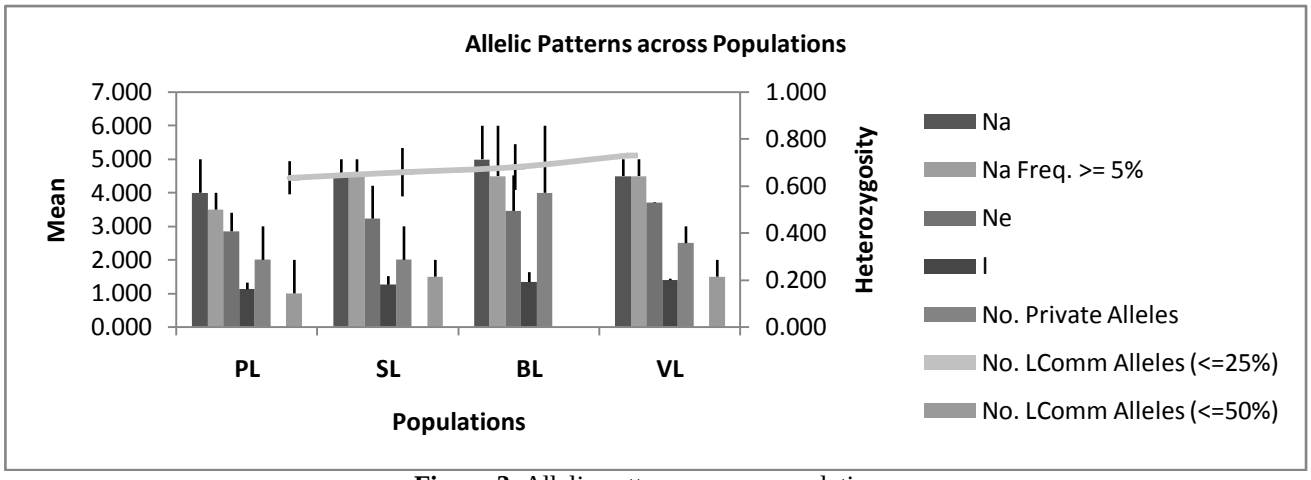


**Figure 3.** Allelic patterns across populations.

Note: 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.

**Genetic variation**

The mean number of effective alleles over all loci and populations is 3.312±0.315 and the average in each population was 2.852±0.553 (Poondi Lake), 3.232±0.979 (Sholavaram Lake), 3.461±1.059 (Bellandur Lake) and 3.704±0.017 (Varthur Lake) as shown in the Table 2. The average number of private alleles detected in population Poondi Lake and Sholavaram Lake were similar (2.000±1.000), Bellandur Lake (4.000±2.000) and Varthur Lake (2.500±0.500).

Observed and expected heterozygosity and homozygosity Nei's heterozygosity and average heterozygosity values for all loci and populations are given in Table 3. The mean of observed heterozygosity (Het_Obs) over two loci and populations; PL is 0.1250±0.0354, SL is 0.1250±0.0354, BL is 0.3000 ±0.3536 and VL is 0.1000 ±0.0707. The mean expected heterozygosity (Het_exp) over loci and populations PL is 0.6519±0.1024, SL is 0.6763 ±0.1496, BL is 0.6987± 0.1414 and VL is 0.7487±0.0018. Observed and expected heterozygosity and homozygosity Nei's heterozygosity and average heterozygosity values for all loci and populations are given in Table 3.

**Hardy-Weinberg equilibrium test**

Measure of HWE test on multilocus based on Hedrick method in GenAIEx V 6.5 showed that 4 population in genetic equilibrium were deviated from equilibrium or highly significant at P<0.001 and Varthur Lake population significant at P<0.01 as shown in (Table 4).

**F Statistics**

The mean $F_{ST}$ was 0.2523 which shows that there is a moderate differentiation between subpopulations. In the present study $F_{IS}$ value were >0.10 (0.7598) indicating heterozygosity deficiency. Average $F_{IT}$ across all loci was 0.8204. The value of Wright's fixation index ($F_{IS}$) is a measure of heterozygote deficiency or excess (inbreeding co-efficient) with an average of 0.7598. F-statistics ($F_{IS}$, $F_{ST}$ and $F_{IT}$) values for each locus are given in the Table 5. The number of migrant's average was reported as 0.7411. Pairwise $F_{ST}$ calculated from two microsatellite loci ranged between 0.160 (Poondi Lake vs. Varthur Lake) to 0.208 (Poondi Lake vs. Sholavaram Lake) and their Nm values for all the populations are given in Table 6.

**Polymorphic Information Content**

The PIC for all population per locus ranged from 0.4824 to 0.7477. The PIC for all populations is given in the Table 7. The BL at locus *Cmac6* showed the high PIC value with 0.7477 and the lowest was observed at PL at locus *Cmac6* with 0.4824.

**Analysis of Molecular Variance (AMOVA)**

To determine whether populations were genetically structured we used analysis of molecular variance (AMOVA). The AMOVA revealed that there was 100% showed high variance with 28%, 56% and 17% variation among populations, among individual and within individual respectively as shown in the Table 8.

**Genetic Distance and Phylogenetic Dendrogram**

Nei's genetic distance (D) values ranged from 0.000 to 5.1778 among the population pair. The value between Poondi Lake and Bellandur Lake population was higher (5.1778) and that between Bellandur Lake and Varthur Lake was lowest (0.000) (Table 9).

The UPGMA dendrogram based on genetic distance resulted in the clusters. Poondi Lake and Sholavaram Lake formed one cluster and; Bellandur Lake and Varthur Lake on the other (Fig. 4).

**Table 3.** Showing heterozygosity statistics for all loci and populations.

| Pop | Locus | Hom_obs | Het_obs | Hom_exp | Het_exp | Nei | Avg_het |
|---|---|---|---|---|---|---|---|
| Poondi Lake | *Cmac*6 | 0.9000 | 0.1000 | 0.4205 | 0.5795 | 0.5650 | 0.6572 |
| | *Cmac*11 | 0.8500 | 0.1500 | 0.2756 | 0.7244 | 0.7063 | 0.6959 |
| Sholavaram | *Cmac*6 | 0.8500 | 0.1500 | 0.4295 | 0.5705 | 0.5563 | 0.6572 |
| Lake | *Cmac*11 | 0.9000 | 0.1000 | 0.2179 | 0.7821 | 0.7625 | 0.6959 |
| Bellandur Lake | *Cmac*6 | 0.4500 | 0.5500 | 0.2013 | 0.7987 | 0.7788 | 0.6572 |
| | *Cmac*11 | 0.9500 | 0.0500 | 0.4013 | 0.5987 | 0.5837 | 0.6959 |
| Varthur Lake | *Cmac*6 | 0.9500 | 0.0500 | 0.2526 | 0.7474 | 0.7288 | 0.6572 |
| | *Cmac*11 | 0.8500 | 0.1500 | 0.2500 | 0.7500 | 0.7312 | 0.6959 |

Expected homozygosity and heterozygosity were computed using (Levene, 1949); (Nei, 1973) expected heterozygosity. Hom_obs= observed homozygosity; Het_obs= observed heterozygosity; Hom_exp= expected homozygosity; Het_exp= expected heterozygosity; Nei= Nei's heterozygosity; Avg_het= Avergeheterozygosity.

**Table 4.** Showing Hardy Weinberg Equilibrium for all populations.

| Pop | Locus | DF | ChiSq | Prob | Signif |
|---|---|---|---|---|---|
| Poondi Lake | *Cmac*6 | 3 | 31.742 | 0.000 | *** |
| Poondi Lake | *Cmac*11 | 10 | 40.527 | 0.000 | *** |
| Sholavaram Lake | *Cmac*6 | 6 | 26.769 | 0.000 | *** |
| Sholavaram Lake | *Cmac*11 | 10 | 62.231 | 0.000 | *** |
| Bellandur Lake | *Cmac*6 | 15 | 30.991 | 0.009 | ** |
| Bellandur Lake | *Cmac*11 | 6 | 40.038 | 0.000 | *** |
| Varthur Lake | *Cmac*6 | 6 | 53.521 | 0.000 | *** |
| Varthur Lake | *Cmac*11 | 10 | 47.756 | 0.000 | *** |

Pop=population; DF- degree of freedom; chisq- chi square; prob-probability value, * P<0.05, ** P<0.01, *** P<0.001

**Table 5.** Summary of F-statistics and gene flow for all loci.

| Locus | Sample Size | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | Nm |
|---|---|---|---|---|---|
| *Cmac*6 | 160 | 0.6767 | 0.7670 | 0.2794 | 0.6449 |
| *Cmac*11 | 160 | 0.8383 | 0.8747 | 0.2247 | 0.8625 |
| Mean | 160 | 0.7598 | 0.8204 | 0.2523 | 0.7411 |

Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST,}$ Nei [31].

**Table 6.** Showing Fst and Nm values for all four populations.

| Pop1 | Pop2 | Fst | Nm | #Pop1 | #Pop2 |
|---|---|---|---|---|---|
| Poondi Lake | Sholavaram Lake | 0.208 | 0.954 | 20 | 20 |
| Poondi Lake | Sholavaram Lake | 0.205 | 0.969 | 20 | 20 |
| Sholavaram Lake | Sholavaram Lake | 0.196 | 1.023 | 20 | 20 |
| Poondi Lake | Varthur Lake | 0.160 | 1.308 | 20 | 20 |
| Sholavaram Lake | Varthur Lake | 0.163 | 1.282 | 20 | 20 |
| Sholavaram Lake | Varthur Lake | 0.174 | 1.191 | 20 | 20 |

**Table 7.** Showing polymorphic information content for two loci and four populations.

| Locus | Populations | | | |
|---|---|---|---|---|
| | PL | SL | BL | VL |
| Cmac6 | 0.4824 | 0.4947 | 0.7477 | 0.6807 |
| Cmac11 | 0.65 | 0.7243 | 0.5242 | 0.6875 |

L- Poondi Lake; SL-Sholavaram Lake; BL- Bellandur Lake; VL- Varthur Lake.

**Table 8:** Showing Analysis of Molecular Variance (AMOVA).

| Source | df | SS | MS | Est. Var. | % |
|---|---|---|---|---|---|
| Among Pops | 3 | 36.519 | 12.173 | 0.273 | 28% |
| Among Individual | 76 | 95.250 | 1.253 | 0.545 | 56% |
| Within Individual | 80 | 13.000 | 0.163 | 0.163 | 17% |
| Total | 159 | 144.769 | | 0.981 | 100% |

Df-degree of freedom; SS-sum of squares; MS-mean squares; Est.var. -Estimated variation; %- percentage of molecular variance.

**Table 9.** Showing genetic distance for four populations.

| Pop ID | PL | SL | BL | VL |
|---|---|---|---|---|
| PL | **** | 0.0563 | 0.0056 | 0.1804 |
| SL | 2.8769 | **** | 0.0068 | 0.1232 |
| BL | 5.1778 | 4.9887 | **** | 0.0000 |
| VL | 1.7124 | 2.0943 | 0.0000 | **** |

Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal).

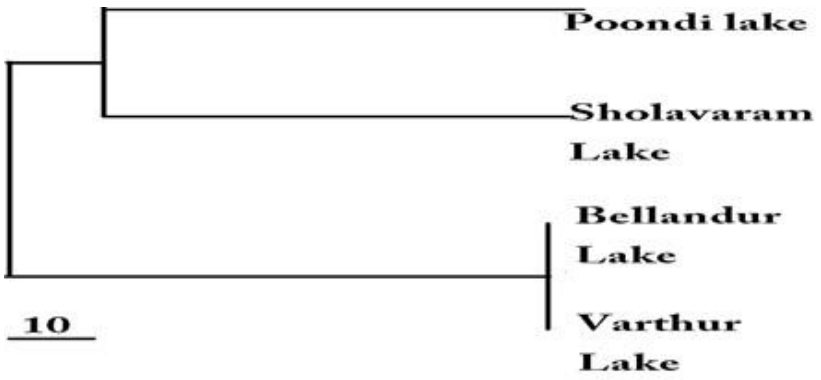


**Figure 4.** Phylogenetic Tree showing the genetic distance between four populations.

## DISCUSSION

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 two primers from *C. macrocephalus* and evaluated for cross species amplification of microsatellite loci in *C. gariepinus.* Both

the loci (*Cmac11* and *Cmac06*) were amplified successfully and resulted in clearly scorable bands on the standard Agarose gel and visualized under Gel analyzer. Cross amplification of primers in *C. gariepinus* shows the evidence of remarkable evolutionary conservation of microsatellite flanking regions (MRFs). This study adds up to the fact that they are closely related to each other. These Siluriformes primers would be helpful even to document the evolution of microsatellites contained in these loci to generate phylogenetic relationships across different species of this order, in addition to their application as potent markers in stock identification of Clarias species

**Cross amplification of microsatellite primers in *C. gariepinus***

In our present study the alleles in Cmac6 ranged from 3-6 and Cmac11 ranged from 4-5 with a size range from 153-215 and 170-210 respectively. In this study *Cmac*6 of Bellandur Lake showed the highest polymorphism and least in Poondi Lake with 3 alleles. 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). Similar study was reported from Bangkok, Thailand by Sukkorntong *et al.* (2008) where they have used very same loci *Cmac6* with 20 alleles and *Cmac*11with only one allele and the size range were from 256-268 and 162 respectively. This is proving polymorphism in *C. gariepinus* using *C. macrocephalus* primers. Mohindra *et al.* (2007) made a comparative study among the species of Clarias using allozyme and mitochondrial DNA markers. In their study with allozyme markers, shows 19.51% sharing between *C.macrocephalus* and *C. gariepinus*. Proving similarity between these two species. Nazia and Azizah (2014) isolated microsatellite from *C. macrocephalus* and cross amplified in *C.batrachus*, *C. meladerma*, *C. gariepinus* and *C. nieuhofii*. In their study the number of alleles per locus in *C. macrocephalus* ranged from 2 to 21 and in *C. gariepinus* alone it ranged from 1 to 3. Thus the result shows that these two species are sharing more common loci.

**Genetic Variation**

Heterozygosity is an important evolutionary indicator in determining the dynamics and survival of populations (Reed, 2009). Information obtained from microsatellite markers showed high genetic diversity among individuals and low diversity within individual. However, low observed heterozygosity was indicative of intra-population genetic structuring of *C.gariepinus* populations. 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 Sukkorntong *et al.* (2008) Cmac6 and Cmac11 observed heterozygosity is 0.7333 and 0.4667 respectively; expected heterozygosity is 0.7480 and 0.4271 respectively. In the Nazia and 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 amplification of primers in related species indicates that the flanking sequences are conserved. This is in agreement with studies on the Pangasius (Na-Nakorn *et al.*, 2006).

**Hardy-Weinberg Equilibrium (HWE)**

At locus Cmac6 Bellandur Lake alone is significant at $P<0.01$. In a similar study by Nazia and Azizah (2014) at the locus NCm-F8 it significantly deviated from HWE ($P<0.05$). Hardy Weinberg equilibrium is based on the random mating in a population; deviations from HWE in wild populations are expected (Dixon *et al.*, 2008). In Sukkorntong *et al.* (2008) in their studies on Cmac6 with a P value of 0.3835 and Cmac11 with 0.6871 indicates no significant deviation from HWE. This study was done in Thailand. But when comparing the same loci in India, it is giving significant value. In Na-Nakorn *et al*.(1999) in *C. macrocephalus* amplified with four *C. macrocephalus* primers, showed homozygote excess as indicated by higher expected heterozygosity than observed.

**F Statistics**

Gametal correlation co-efficient (FST) 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) FST value less than 0.05 indicate the low differentiation among communities; hence the results represent high between populations. Wright's fixation index (FIS), a measure of the inbreeding coefficient, was in agreement with the other results, being low when genetic diversity was high and medium or high when the heterozygosity was low. Van Oosterhout *et al*. (2006) excess homozygosity can be due to deviation from panmixia, inbreeding short allele dominance. Li *et al.* (2009) notes that when $Nm>1$ and $Nm<1$, then genetic differentiation occurred due to number of migrant and gene migrant respectively and gene flow or effective migration value (Nm) for each locus were 0.6449 (Cmac6) and 0.8625 (Cmac11). The overall gene flow (Nm) among the populations over all loci was 0.7411 this indicated that Nm might be the significant factor between populations. That is gene flow was the main factor for genetic differentiation.

**Polymorphic Information Content**

In the study of Nazia and Azizah (2014) the PIC per locus ranged from 0.032 to 0.92, while in our present study per locus ranged from 0.4824 to 0.7477. Markers with PIC values >0.4 are considered moderately informative and those with values >0.7 are considered highly informative (Hildebrand *et al.*, 1992). In the present study all the values are $\geq 0.4$ and $\leq 0.7$ indicating they are moderately informative due to the low sample number used. These parameters indicate that the four populations belonged to the level of high polymorphism and genetic variations were also high.

**Analysis of Molecular Variance (AMOVA)**

In our study the AMOVA revealed that there was 100% variance. High genetic variation between and within populations indicate utilization of natural populations for either genetic improvement program or for aquaculture. In the present study the stock was 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).

**Genetic Distance and Phylogenetic Dendrogram**

The phylogenetic dendrogram constructed from Nei's genetic distance also shows the separation of Chennai from Bangalore populations (Figure 4). The UPGMA pattern from the two markers helped to identify the distance between the four populations. In the study of Mohindra *et al.* (2007) it was identified that markers were helpful to discriminate the native *C. batrachus* from *C. gariepinus* and *C. macrocephalus.*

## CONCLUSIONS

The successful cross-species amplification of *C. macrocephalus* microsatellite loci over *C. gariepinus* described herein can be attributed to the high conservatism of the flanking microsatellite regions. First, the potential use of heterologous primers was explored and they found to be conserved in this species. Second, microsatellite loci used were polymorphic and showed heterogeneity in allele frequency in all four population. Third, the study suggested that these four natural populations of this species, viz., Poondi Lake, Sholavaram Lake, Bellandur Lake, and Varthur Lake are divergent in their genetic characteristics.

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