Genetic variation and differentiation in African catfish, *Clarias gariepinus*, assessed by heterologous microsatellite DNA

P Ezilrani and J Godwin Christopher*
Molecular and Microbiology Research Laboratory, Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore 632 014, India

Received 17 February 2014; revised 5 October 2014; accepted 28 November 2014

The population structure and genetic variation between *Clarias gariepinus* populations collected from 3 different regions of South India (Vellore, Chennai & Bangalore) were analyzed using cross species amplification of microsatellite markers developed from *C. batrachus* and *C. macrocephalus* (Cba12, Cha17, Cmac6 & Cmac11). Samples were amplified using polymerase chain reaction (PCR). Fifty four alleles were recorded with 3 to 6 alleles per locus. Allelic frequency (Pi), observed heterozygosity (He obs), expected heterozygosity (He exp), polymorphism information content (PIC) and effective alleles were determined. Hardy-Weinberg equilibrium was checked according to the chi square (χ²) test and were significant at P<0.001. Genetic differentiation and genetic diversity were evaluated by gametal correlation coefficient (Fst) and gene flow (Nm). The loci were polymorphic in all the populations. The clustering dendrogram was made based on the results of UPGMA methods using POPGENE software, which revealed the distance between three population forming two clusters; one based on Vellore and another cluster based on Chennai and Bangalore populations. Thus the present study will be useful for the analysis of population genetic diversity, and the management of this important fish resource.

**Keywords:** *Clarias gariepinus*, cross-amplification, genetic distance, genetic variability, microsatellite

**Introduction**

The catfish genus *Clarias* has been found in both the Asian and African continents. The species *C. gariepinus* is a freshwater fish, widely tolerant of extreme environmental conditions. It is an air breathing catfish, which makes it to be sold live in the market. The features that make this species a potential candidate for aquaculture include fast growth rate, hardiness, efficient feed utilization and the ability to survive in poorly oxygenated waters. It is being cultured commercially almost everywhere. As this species is cultured in all continents, it’s expected that it could have acquired more genetic variation.

The genetic variation in a species helps it to adapt to the changing environmental conditions. This variation in a gene may occur because of the spontaneous mutation or by migration from population of genetically different individuals. The amount of genetic variation occurring within and between populations can be determined by microsatellite DNA markers. Microsatellites or simple sequence repeats are highly polymorphic in nature comprising of mono-, di-, tri- or tetra-nucleotides that are repeated in tandem arrays. They are widely distributed throughout the genome, which makes suitable the use of this microsatellite region for genetic studies. However, microsatellites are not always available for the species to be studied and their isolation could be time-consuming. Hence, researchers often rely on cross-species amplification. Microsatellites DNA are easy to score by polymerase chain reaction (PCR) and widely used for the study of linkage mapping, comparative mapping, demographic structure and phylogenetic history in populations.

Microsatellite markers have been isolated from several *Clarias* catfishes including *C. batrachus*, *C. macrocephalus*, and *C. gariepinus*. In the present study, microsatellite DNA markers developed by Sukkorntong et al. from *C. macrocephalus* and by Yue et al. from *C. batrachus* were used to reveal and compare the genetic structure of three South Indian populations (Vellore, Chennai and Bangalore) of *C. gariepinus*.

**Materials and Methods**

**Sample Collection and DNA Extraction**

A sample of 20 specimens of *C. gariepinus* were purchased from Chennai, Vellore and Bangalore fish markets as live fish (Fig. 1). They were transported to...
VIT University in simple plastic pots. These live fish were anesthetized with MS 222 and approx 300 mg of caudal fin tissues were removed from each individual. The fin tissues were cut into small pieces with scissors and ground with digestion buffer in a tissue grinder in a 1.5 mL glass homogenizer.

Genomic DNA was isolated from fin tissue samples using the protease K digestion and phenol-chloroform extraction method. Purified DNA was quantified and stored at −20°C. The quality of DNA was tested by electrophoresis on 1% agarose gel and the quantity was determined by using a spectrophotometer (Eppendorf).

**Amplification of Microsatellite Loci**

Four primers, Cba12, Cba17 (developed by Yue et al.), Cmac6 and Cmac11 (developed by Sukkorntrong et al.) were used for PCR amplification in the study. Their details are given in Table 1.

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 MgCl₂. Thermal profiles of reaction are given in Table 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 50 bp standard DNA ladder (GENEI Pvt. Ltd., Bangalore, India), and the bands were analyzed using DNA Alpha view software 3.3.1 (www.cellbiosciences.com; http://www.proteinsimple.com/accessories_alphaimager.html).

**Statistical Analysis of Microsatellite Data**

Genotype of each individual fish was determined and recorded from the gels for each microsatellite locus. Size of the bands representing particular alleles at the microsatellite loci were estimated using the DNA Alpha view software 3.3.1.0. Scoring of genotypes for a specific locus was performed on the basis of the number of alleles of a particular size present in that locus. A single genotypic data matrix was constructed for all loci. Estimation of allelic variations, effective number of alleles, heterozygosity values (observed and expected), deviation from Hardy-Weinberg equilibrium, fixation index, Analysis of molecular variance (AMOVA) were performed using the software GenAIEx 6.5. Population differentiation (Fst) values and gene flow

---

**Table 1—Showing the details of four microsatellite primers**

<table>
<thead>
<tr>
<th>No.</th>
<th>Primer sequence (5’→3’)</th>
<th>Locus</th>
<th>Ann. temp. (°C)</th>
<th>Clone acc. no.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F- ACGCCGGCTGTGTTTTCATCTCC R- ACAACGCTGAATCCAGGGGCA</td>
<td>Cba12</td>
<td>59</td>
<td>AY169267</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>F- ACACTCCAGTGCAACGAGGCA R- TGCACACCAGCAGTAACACGAG</td>
<td>Cba17</td>
<td>58.1</td>
<td>AY169270</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>F- GCACAGTGTCAAGGCTTCTGC R- TGTGTGTGATGTGGTACAGCC</td>
<td>Cmac11</td>
<td>56.6</td>
<td>EU179753</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>F- GCACAGGAGGGGAGACTGACGA R- TGGGCACAGGACATCGAAGCT</td>
<td>Cmac6</td>
<td>56.6</td>
<td>EU179743</td>
<td>10</td>
</tr>
</tbody>
</table>

---

**Table 2—Showing PCR conditions**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Temp (°C)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94</td>
<td>5 min</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94</td>
<td>30 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>Gradient (Table 1)</td>
<td>30 sec</td>
</tr>
<tr>
<td>Initial elongation</td>
<td>72 (30 cycles)</td>
<td>1 min</td>
</tr>
<tr>
<td>Elongation</td>
<td>72</td>
<td>10 min</td>
</tr>
</tbody>
</table>
was performed using POPGENE V 1.32\textsuperscript{17}. Homozygosity (observed and expected) by GENEPOP 4.1.4\textsuperscript{18} and polymorphic information content (PIC) was detected using Microsatellite tool kit\textsuperscript{19}. A dendrogram was drawn based on the Nei’s genetic distance\textsuperscript{17} between the populations following unweighted pair group method of averages (UPGMA) using the software POPGENE V 1.32\textsuperscript{17}. TREEVIEW\textsuperscript{20} software was used to obtain the bootstrap value and construct to the tree.

Results and Discussion

Genetic variation is an important foundation for evaluating species resources. It reflects how the species adapts to various environmental changes. Thus the genetic structures of a fish species is subject to change over time under some biological and physical factors.

PCR Amplification

All four microsatellite loci (Cba12, Cba17, Cmac11 & Cmac6) were successfully amplified in all the three populations. In total, 240 fragments ranging from 98 to 343 bp in length were amplified. The number of alleles per locus was 3 to 6; in total 54 alleles, 13.5 on an average as shown in Table 3. Observed heterozygosity was not seen in any population. The allelic frequency is shown in the (Fig. 2). Microsatellite primers developed for one species sometimes cross-amplify microsatellite loci in closely related species\textsuperscript{21}. With a view to studying cross-species amplification, we tested four pairs of primers (Cba12, Cba17, Cmac6 & Cmac11) developed for *C. batrachus* and *C. macrocephalus*\textsuperscript{10,11} in *C. gariepinus* to analyze genetic structure. All the

![Fig 2—Allele frequencies by population and locus for codominant data of *C. gariepinus*. (popvel: Population Vellore; popche: Population Chennai; popbang: Population Bangalore)](image-url)

| Table 3—Showing polymorphic information of the four microsatellite loci in *C. gariepinus* of different populations |
|---|---|---|---|---|---|---|---|---|---|
| Population | Locus | Size range (bp) | Na | Ne | I | Ho(obs) | Ho(exp) | Ho | He | PIC | F | TNG |
| Vellore | Cba12 | 330-343 | 5.000 | 4.651 | 1.574 | 20 | 3.8974 | 0.000 | 0.785 | 0.75 | 1.000 | 15 |
| | Cba17 | 98-115 | 6.000 | 5.263 | 1.709 | 20 | 3.3846 | 0.000 | 0.810 | 0.78 | 1.000 | 21 |
| | Cmac6 | 150-161 | 4.000 | 3.922 | 1.376 | 20 | 4.7179 | 0.000 | 0.745 | 0.69 | 1.000 | 10 |
| | Cmac11 | 196-223 | 4.000 | 3.509 | 1.305 | 19 | 5.2564 | 0.000 | 0.715 | 0.66 | 1.000 | 10 |
| Chennai | Cba12 | 300-317 | 4.000 | 3.704 | 1.345 | 20 | 5.0256 | 0.000 | 0.730 | 0.68 | 1.000 | 10 |
| | Cba17 | 102-120 | 5.000 | 4.255 | 1.522 | 20 | 4.3077 | 0.000 | 0.765 | 0.72 | 1.000 | 15 |
| | Cmac6 | 153-170 | 5.000 | 4.082 | 1.483 | 19 | 4.3333 | 0.000 | 0.755 | 0.72 | 1.000 | 15 |
| | Cmac11 | 180-193 | 5.000 | 5.000 | 1.609 | 20 | 3.5897 | 0.000 | 0.800 | 0.76 | 1.000 | 15 |
| Bangalore | Cba12 | 297-309 | 4.000 | 3.922 | 1.376 | 20 | 4.7179 | 0.000 | 0.745 | 0.69 | 1.000 | 10 |
| | Cba17 | 115-130 | 5.000 | 4.651 | 1.567 | 20 | 3.8974 | 0.000 | 0.785 | 0.74 | 1.000 | 15 |
| | Cmac6 | 157-164 | 3.000 | 2.597 | 1.010 | 20 | 7.3846 | 0.000 | 0.615 | 0.53 | 1.000 | 6 |
| | Cmac11 | 185-210 | 4.000 | 3.774 | 1.354 | 20 | 4.9231 | 0.000 | 0.735 | 0.68 | 1.000 | 10 |

Na: No. of average alleles; Ne: No. of effective alleles; I: Shannons information index; Ho (obs): Observed homozygosity; Ho (exp): Expected homozygosity; Ho: Observed heterozygosity; He: Expected heterozygosity; PIC: Polymorphic information content; F: Fixation index; TNG: Total no. of genotypes
four markers tested cross amplified successfully in *C. gariepinus*, indicating some degree of conservation of primer sites between *C. gariepinus* with its closely related freshwater catfish species of *C. batrachus* and *C. macrocephalus*, as far as these loci are concerned. Similar type of cross species amplification was observed in *Heteropneustes fossilis* while using *C. batrachus* primer. However, of 5 different primers used, only 2 primers successfully cross amplified. This shows the genetic closeness between *H. fossilis* and the *C. batrachus*. Further, Agbebi et al. used four *C. gariepinus* microsatellite markers (*Cga1, Cga2, Cga3 & Cga5*) to study heterozygosity in *Heterobranchus bidorsalis* by cross amplifying these loci.

**Genetic Variation in Population**

In the three populations of *C. gariepinus*, the effective alleles ranged from 2.59 to 5.26 and the average alleles in each population were 4.34 (Vellore), 0.76 (Chennai) and 0.72 (Bangalore). The number of private alleles detected in Vellore population was 4.00, while 3.75 were in Chennai and 2.50 in Bangalore. The sizes and length of amplified alleles might not be the same as those of the homologous species, e.g., the size of the alleles at locus *Cba12, Cba17, Cmac6* and *Cmac11* ranged from 297-343 bp. But when the same primer was used in *C. batrachus* it amplified different base pair length in homologous species; *Cba12* amplified allele size of 130 and 132 bp in homologous amplification. This reveals the difference in genetic structure.

Polymorphic information contents (PIC) of the populations varied between 0.53 to 0.78 and their averages were 0.72 (Vellore), 0.72 (Chennai) and 0.66 (Bangalore) in each population (Table 3). The PIC value was higher than 0.5 for all the populations, which indicates that *C. gariepinus* populations have high polymorphism as well as high genetic variation. A similar analysis was done by Agbebi et al. in *C. gariepinus* using *H. bidorsalis* primer. The PIC value depend on the number of alleles detected per locus and their frequencies; as higher number of alleles are recorded, its a better genetic population.

**Hardy-Weinberg Equilibrium (HWE) Test**

Measure of HWE test on multilocus, based on Hedrick method in GenAIEx V 6.5, showed that all the three populations of *C. gariepinus* were in genetic equilibrium and the data were highly significant at *P*<0.001 as shown in (Table 4). Since the populations are generally from the hatchery and their parents are not from the wild stock, such deviation could be a result of one or more of the following factors, such as, evolutionary forces, non-random mating, and random changes in allele frequency in a population, mutation, migration and natural selection. A similar study by Agbebi et al. in *C. gariepinus* revealed homozygosity for the population at loci *Cga2* and *Cga5*.

**Genetic Differentiation in Population**

Gamatal correlation coefficient (Fst) also known as co-efficient of inbreeding and gene flow (Nm) were computed to estimate the differences between population. The Fst was found to be 0.1622, which shows that there is a moderate differentiation between subpopulations (Table 5). The overall gene flow (Nm) among the populations over all loci was 1.2909, this indicates that it is a significant factor between populations and gene flow is the main factor for
genetic differentiation. Total number of genotypes ranged from 6 to 21. The fixation index values were 1, indicating inbreeding or undetected null alleles in these populations.

Based on the analysis of molecular variance (AMOVA) summarized in Table 6, genetic variation in this populations was mainly attributable to individual-level variation, with a percentage of variance of 82%. Variation between fish within populations represented 18% of the variation, while it was negligible to 0% within populations. The catfish origin (hatchery) and also the cultivation practices could have been responsible for the low level of genetic differentiation.

**Genetic Distance and Phylogenetic Dendrogram**

Nei’s genetic distance (D) values ranged from 1.5798 to 3.4526 among the pair population (Table 7). The value between Vellore and Chennai population was the highest (3.4526), while that between Chennai and Bangalore was the lowest (1.5798). The UPGMA dendrogram based on genetic distance resulted in the two clusters. Vellore population alone was in one cluster and the other two Chennai and Bangalore in the other clusters (Fig. 3). This shows that the Vellore population is distinct from that of Chennai and Bangalore population, while Chennai and Bangalore populations are similar. The phylogenetic tree across population shows separation of the species into clade with a bootstrap value of 53. The bootstrap value shows the genetic distance between the populations. Similar type of dendrogram analysis was also performed in different populations of *H. fossilis* in Bangladesh.

The microsatellite markers are powerful tool for fish identification. Genetic monitoring is necessary for an effective management strategy. The microsatellite technique has been found suitable for characterizing the genetic structure of this African catfish between three different places. It’s found that Chennai and Bangalore are more genetically similar than Vellore catfish. This shows that the similar population might had similar origin of broodstock form hatchery.

**Acknowledgement**

The authors express sincere thanks to the Management and Staff of VIT University, Vellore for providing necessary facilities to carry out this research.

**Reference**


