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# CHARACTERIZATION OF *CHANNA MARULIUS* POPULATIONS IN VARIOUS WATER BODIES OF PUNJAB, PAKISTAN

**Ehsan Mehmood Bhatti[1], Naureen Aziz Qureshi[1], Fayyaz Rasool[2,*], Muhammad Siddique[3] and Abu Baker Siddiqui[1]**

**[1]Department of Wildlife and Fisheries, Faculty of Sciences and Technology, GC-University Faisalabad, Pakistan; [2]Department of Fisheries and Aquaculture, Faculty of Fisheries and Wildlife, University of Veterinary and Animal Sciences, Lahore, Pakistan; [3]Department of Environmental Sciences, Faculty of Sciences and Technology, GC-University Faisalabad; Pakistan.**
**[*]Corresponding author's e.mail: drfayyaz1980@gmail.com**

In Pakistan the work on genetic of different populations of *Channa marulius* have not been performed so for and the Clustering analysis was done to find out the intraspecific variations among different populations of *Channa marulius* using Pearson Correlation Coefficient method based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) by XLSTAT 2012 (version 1.02). 50 samples of *C. marulius* populations were collected from five different geographical sites of natural waters viz., Chashma Barrage at River Indus (District Mianwali), Qadirabad Barrage at the River Chenab (District Gujranwala), Balloki Barrage at River Ravi (District Kasur), Trimmu Barrage at the junction of Chenab and Jhelum Rivers (District Jhang) and Taunsa Barrage at Indus River (Tehsil Kot Addu District Muzaffar Garh). Dendrogram generated by RAPD data of representative samples of each site divided the populations of *C. marulius* into four major clusters. The results of the studies clearly indicate that variance decomposition for the optimal classification values remained as, 65.90% for within class variation while 34.10% for the between class differences. The distance between the class/cluster centroids was 1.737 for class 1 and 2, 1.105 for 1 and 3, 1.009 for 1 and 4, 1.500 for class 2 and 3, 2.000 for 2 and 4, while this distance between class 3 and 4 was 1.500. Quantifiable differentiation among various species of *C. marulius* revealed the impacts of changing environment and other possible factors.

**Keywords:** Clustering, morphometeric, intraspecific, variations, *Channa marulius*

## INTRODUCTION

The Family Channidae consists of two genera viz., *Channa* and *Parachanna* (Nelson, 1984). The Asian genus *Channa*, presently contains 26 valid species, is widely distributed in Iran, southern Asia and the Far East (Musikasinthorn, 2000; Berra, 2001; Musikasinthorn and Taki, 2001; Courtenay and Williams, 2004) and have been reported even at an altitude of 475 m above mean sea level (Munro, 1955). Members of this group commonly inhabit rivers, swamps, ponds, canals, drains, reservoirs, rice fields etc. of southern Asia, southern China, Indo-China and Sunda Islands (Mohsin and Ambak, 1983; Lee and Ng, 1994; Hossain *et al.*, 2008). In Pakistan, these fishes are commonly harvested from natural freshwater bodies and are sold alive due to consumer preferences (Mirza, 1982; Rahman, 1989). In Thailand, Taiwan, the Philippines, Vietnam, Malaysia, Cambodia and India, their production significantly contributes to total fish catch (Wee and Tacon, 1982; Hossain *et al.*, 2008; Jamaludine *et al.*, 2011). The African genus *Parachanna* contains three valid species, which are restricted to West Africa (Bonou and Teugels, 1985; Teugels, 1992). Amongst the snakeheads, *C. marulius* (Hamilton, 1822) and *C. punctatus* (Bloch, 1793) are commercially important esteemed table fish and also used for ornamental trade (Ng and Lim, 1990; Courtenay and Williams, 2004). The members of genus *Channa* (snakehead, murrel) are prominent tropical freshwater fishes and are widely utilized for medicinal and pharmaceutical purposes (Mat-Jais *et al.*, 1994; Michelle *et al.*, 2004; Zafar *et al.*, 2012), in addition to their consumption as food in Asia-Pacific region (Hossain *et al.*, 2008).

Murrels has long cylindrical body, long and entirely soft-rayed dorsal and anal fins, a large mouth with well-developed teeth on both upper and lower jaws. It has been observed that some Asian species entirely lack pelvic fins (Zhang *et al.*, 2002; Musikasinthorn, 2003). In addition to its normal breathing system it also has an accessory air-breathing apparatus called suprabranchial organ which helps under conditions of extreme Oxygen (Musikasinthorn, 1998, 2003). *C. marulius* is the fastest growing species among murrels reaching a length of 120–122 cm (Bardach *et al.*, 1972; Talwar and Jhingran, 1992). In polyculture system, this fish is also used as police fish to control the number of young tilapia in a fish farm (Curz and Laudenica, 1980; Khan *et al.*, 2012). In Indus River and its tributaries, significant research work has been done on its ichthyodiversity (Ahmad,1960, 1963; Banarescu and Nalbant, 1966; Butt and Butt, 1988; Hussain, 1973; Qureshi, 1965; Salam *et al.,* 1997; Urooj *et al.,* 2011).

With the decreasing flows of Indus River system and ecological changes due to extended dry periods combined with overexploitation have led to decrease in fisheries resources. Very little work was done on the differentiation of stocks and populations based on either morphometric or molecular approaches in Pakistan. There is an urgent need to understand the changes in genetic diversity of the fish populations and stocks of Indus river system. The paucity of literature in this field necessities the study of genetic diversity of the fish stocks/ populations in the major rivers of the country. Keeping in view the current scenario these studies were planned to examine the status of distinction of the representatives of the family, Channidae *Channa marulius*, due to some environmental changes and other unknown factors in natural reservoirs.

## MATERIALS AND METHODS

The samples of *Channa marulius* were collected from Chashma Barrage at River Indus (District Mianwali), Qadirabad Barrage at the River Chenab (District Gujranwala), Balloki Barrage at River Ravi (District Kasur), Trimmu Barrage at the junction of Chenab and Jhelum Rivers (District Jhang) and Taunsa Barrage at Indus River (Tehsil Kot Addu District Muzaffar Garh) (Fig. 1 and 2).

Gel electrophoresis was performed after the Polymerase Chain Reactions and amplification of the total genomic DNA extracted from the samples of *C. marulius* collected from all study sites and the data for scorable amplified bands was organized on the one-zero pattern i.e. the presence of band recorded as "1" and for absence marked as "0". To investigate the genetic similarity within and between the populations of *C. marulius* collected from the different sites of study in the same cluster/class from the different geographical locations, Jaccard's coefficient matrix method was used to generate Agglomerative Hierarchical Clustering (AHC) by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) by XLSTAT 2012 version 1.02.

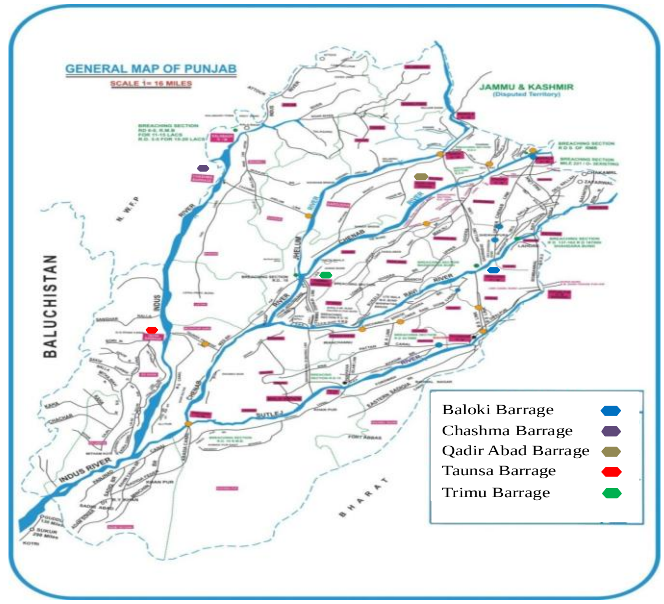


**Figure 1. Map showing the sampling sites at different rivers in Punjab-Pakistan**

**Figure 2. *Channa marulius* (Saul)**

## RESULTS AND DISCUSSION

The RAPD marker used were analyzed for their share in polymorphism as indicated in Table 1. The amplified PCR products were analyzed by gel electrophoresis which is shown in Fig. 3, 4 and 5. A dendrogram showing clustering of genotypes based on amplification of the most scorable bands in random samples and dendrogram of the four separated classes is given in Figs. 6 and 7.

**Table 1. Percentage polymorphic hare of the used RAPD**

| Sr. No. | Name of Primers | Sequence of the primers | No. of total amplified bands | No. of total monomorphic bands | No. of total polymorphic bands | Polymorphism (%) |
|---|---|---|---|---|---|---|
| 1 | OPB-02 | TGATCCCTGG | 5 | 2 | 3 | 60.00 |
| 2 | OPB-06 | TGCTCTGCCC | 6 | 2 | 4 | 66.67 |
| 3 | OPC-11 | AAAGCTGCGG | 6 | 5 | 1 | 16.67 |
| 4 | OPC-13 | AAGCCTCGTC | 5 | 4 | 1 | 20.00 |
| 5 | OPC-15 | GACGGATCAG | 7 | 6 | 1 | 14.29 |
| 6 | OPD-01 | ACCGCGAAGG | 5 | 4 | 1 | 20.00 |
| 7 | OPD-02 | GGACCCAACC | 4 | 3 | 1 | 25.00 |
| 8 | OPD-03 | GTCGCCGTCA | 7 | 6 | 1 | 14.29 |
| 9 | OPD-04 | TCTGGTGAGG | 7 | 2 | 5 | 71.43 |
| 10 | OPD-05 | TGAGCGGACA | 3 | 1 | 2 | 66.67 |

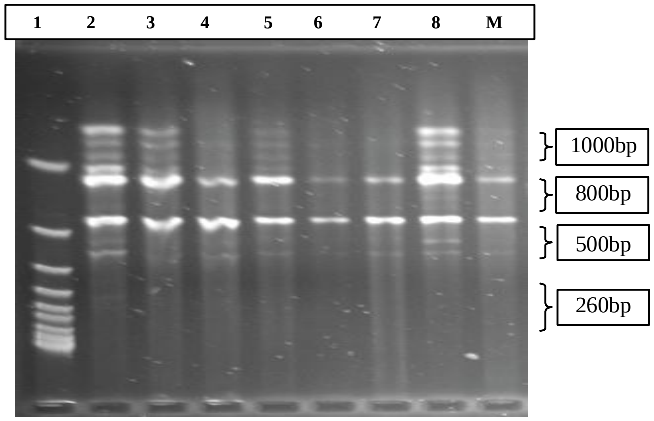


**Figure 3. Picture showing the amplification of the OPB-2 for the samples collected from Baloki**

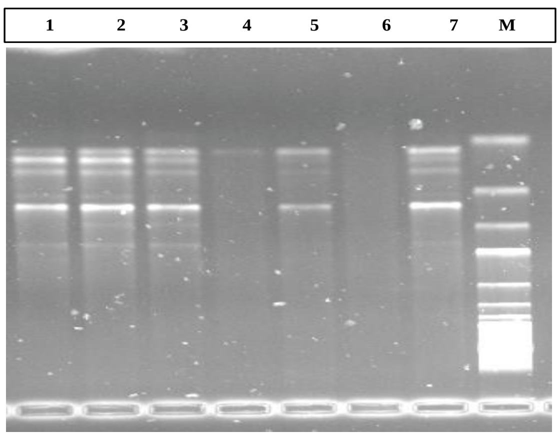


**Figure 4. Picture showing the amplification of OPD-4 for the samples collected from Baloki**

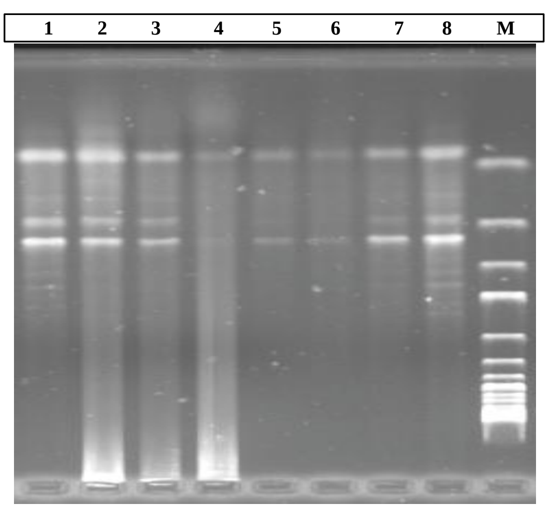


**Figure 5. Picture showing the amplification of OPD-4 samples collected from Qadirabad**

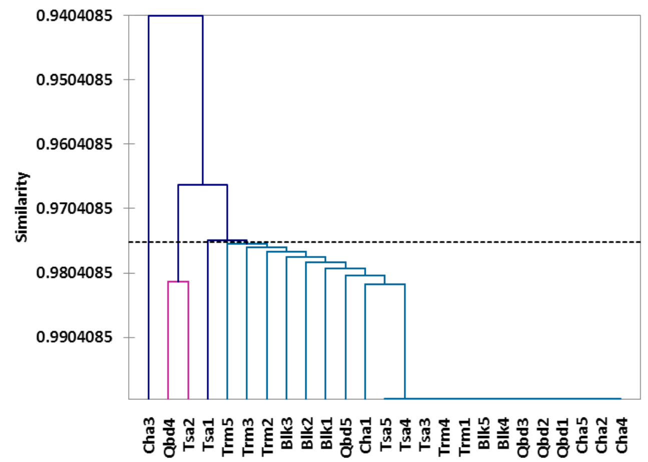


**Figure 6. Dendrogram showing classification of *Channa marulius***

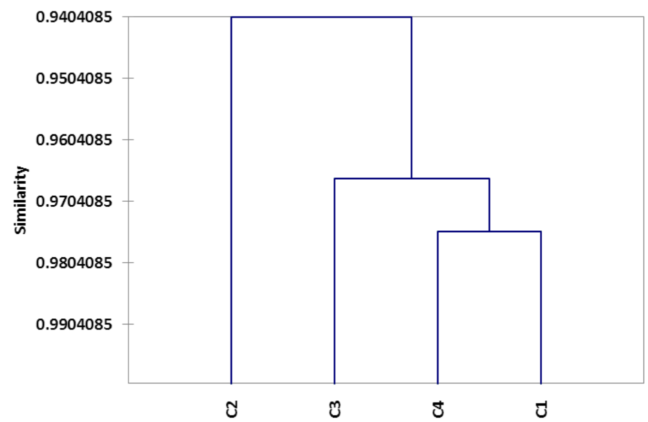


**Figure 7. Dendrogram showing classes of *C. marulius***

The variance decomposition for the optimum classification values remained as 65.90% for within class variation and 34.10% for the between class differences (Table 2) The distance between the class/clusters centroids remained as 1.737 for class 1 and 2, 1.105 for class 1 and 3, 1.009 for class 1 and 4, 1.500 for class 2 and 3, 2.000 for class 2 and 4, while this distance was 1.500 for class 3 and 4 (Table 3). In this classification Cha2 from the Samples collected from Chashma Barrage, Cha3 collected from the same location, Qbd4 collected from Qadirabad Barrage and Tsa1collected from Taunsa Barrage at River Indus were central objects for class/clusters 1, 2, 3 and 4, respectively. The distances between the central objects of the classes remained as; 1.732 between the central objects of class 1 and 2, 1.414 between the class 1 and 3, 1.000 between the central objects of class 1 and 4, 1.732 was the distance between central objects of class 2 and 3, 2.000 was the distance between central objects of class 2 and 4, while this distance for central objects of class 3 and 4 was also 1.732 (Table 4).

**Table 2. Variance decomposition for the optimal classification of *C. marulius***

| | Absolute | Percent |
|---|---|---|
| Within-Class | 0.387 | 65.90% |
| Between-Class | 0.200 | 34.10% |
| Total | 0.587 | 100.00% |

**Table 3. Distances between the class centroids of *C. marulius***

| Classes | 2 | 3 | 4 |
|---|---|---|---|
| 1 | 1.737 | 1.105 | 1.009 |
| 2 | - | 1.500 | 2.000 |
| 3 | - | - | 1.500 |

**Table 4. Central objects of the classes of *C. marulius* and distances between them**

| Central objects | 2 (Cha3) | 3 (Qbd4) | 4 (Tsa1) |
|---|---|---|---|
| 1 (Cha2) | 1.732 | 1.414 | 1.000 |
| 2 (Cha3) | - | 1.732 | 2.000 |
| 3 (Qbd4) | - | - | 1.732 |

The dendrogram developed by this method by the presented data of the scorable bands of the all amplified primers divided the randomly selected individuals of the five populations into four classes/clusters. The division of all the randomly selected five population representative *C. marulius* samples collected from different geographical locations in the four clusters was as follows; Blk1, Blk2, Blk3, Blk4, Blk5, Cha1, Cha2, Cha4, Cha5, Qbd1, Qbd2, Qbd3, Qbd5, Trm1, Trm2, Trm3, Trm4, Trm5, Tsa3, Tsa4 and Tsa5 in 1st cluster/class, Cha3 in 2nd cluster/class, Qbd4, and Tsa2 in the 3rd class and Tsa1 in 4th class/cluster (Table 5).

The results for the conclusion about 5 classes/clusters with their values for within class variance, minimum distance to centroids, average distance to centroids and maximum distance to centroids are given in Table 5.

In this arrangement of samples into cluster; Cha2 from the samples collected from Chashma Barrage, Cha3 collected from the same location, Qbd4 collected from Qadirabad Barrage and Tsa1 collected from Taunsa Barrage at River Indus were central objects for class/clusters 1, 2, 3 and 4, respectively. The distances between the central objects of the classes remained as; 1.732 between the central objects of class 1 and 2, 1.414 between the class 1 and 3, 1.000 between the central objects of class 1 and 4, 1.732 was the distance between central objects of class 2 and 3, 2.000 was the distance between central objects of class 2 and 4, while this distance for central objects of class 3 and 4 was also 1.732.

Morphometric comparisons of the African cat fish, *Clarias gariepinus* populations in Turkey was also conducted by Turan *et al.* (2005) and the results are in accordance with the present studies. These results are also in accordance with the Stergiou and Vasiliki (2003) while working on length and girth interrelationship of the fishes of the marine environment, concluded that the said parameters of 18 marine species were significantly different .

The results of present study are in line with the study of Rasool *et al*. (2012). In their studies on Morphometric Parameters in Hatchery Raised and Natural Populations of *Labeo rohita*, they found that the body weight, total length and average length of paired pectoral fins of *L. rohita* were non-significant (p> 0.05), anal fin length was significantly different (P<0.05), and all the remaining parameters were highly significantly different (p<0.01) among the sites. The condition factor and length-weight relationship was also calculated. The correlation of fish body weight showed highly significant (p<0.001) and positive correlation with all the morphometric parameters but the fork length of the *L. rohita* showed a positive and highly significant (p< 0.0001) correlation with all the parameters except with the caudal fin length where the correlation was slightly positive.

The findings of present study are also in accordance with the work of Rasool *et al*. (2012b) on the populations of *Labeo rohita*. In their study on morphometric parameters performed principle component analysis to determine the variation among the populations found that the PCA divided

**Table 5. The variance level amongst the classes of *C. marulius***

| Class | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Objects | 21 | 1 | 2 | 1 |
| Sum of weights | 21 | 1 | 2 | 1 |
| Within-class variance | 0.381 | 0.000 | 0.500 | 0.000 |
| Minimum distance to centroid | 0.135 | 0.000 | 0.500 | 0.000 |
| Average distance to centroid | 0.449 | 0.000 | 0.500 | 0.000 |
| Maximum distance to centroid | 0.961 | 0.000 | 0.500 | 0.000 |
| Individuals in four classes | Cha1, Cha2, Cha4, Cha5, Qbd1, Qbd2, Qbd3, Qbd5, Blk1, Blk2, Blk3, Blk4, Blk5, Trm1, Trm2, Trm3, Trm4, Trm5, Tsa3, Tsa4, Tsa5 | Cha3 | Qbd4, Tsa2 | Tsa1 |

the fish populations of five locations on the basis of morphometery into two main components, which all together accounted for 80.27% of the cumulative variation among the morphometric parameters. The first group amongst the major two groups accounted for 64.25% of the cumulative variability while the second accounted for 16.03% of the cumulative variability. In the same way the cumulative variation has been observed in all the fish species under study as mentioned above.

This research represents an important advancement in understanding biology of commercial important carnivore fishes of the Indus basin and highlights the morphological divergence in this widely distributed *C. marulius* and *Sperata seenghala* populations. Such divergence is commonly observed for freshwater and lacustrine populations (Schluter and Mcphail, 1992; Snorrason *et al.*, 1994). Present research has identified significant differences in the *C. marulius* and *S. seenghala* populations in the five locations studied. If shape is related to either environmental influences on larval development (Cardin and Silva, 2005) or diversifying selection and ecological adaptation at a trophic level (Costa and Cataudella, 2007), then spatially or latitudinally different environmental factors (e.g. temperature and resource availability) may explain the variations in morphometric heterogeneity of these species.

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