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CLUSTERING ANALYSIS FOR INTRASPECIFIC VARIATION STUDIES AMONGST THE POPULATIONS OF *CIRRHINUS MRIGALA*

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ABSTRACT

The agglomerative clustering analysis to find out the intraspecific variations among the populations of *Cirrhinus mrigala* was done by using Pearson Correlation Coefficient based Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as Agglomeration method by XLSTAT 2012 version 1.02. The data on morphometrics of the samples of *C. mrigala* populations were collected from five different geographical sites of the farmed and natural water fish. A dendrogram with the data on the morphometrics of the representative samples of each site divided the populations of *C. mrigala* in to four major clusters or classes. From the results it is clearly indicated that variance decomposition for the optimal classification values remained as, 27.28% for within class variation while 72.72% for the between class differences. The distance between the class/cluster centroids remained as; 50.820 for class one and two, 18.063 for class one and three, 14.564 for class one and four, 68.856 for class two and three, 36.708 for two and four while this distance between class three and four centroids was 32.408. A measurable distinction between the classes of the populations of the *C. mrigala* was indicated in this study which determined the impacts of changing environment and other possible factors influencing the intraspecific variation among the populations of the same species.

Key words: Intraspecific**,** AHC, *Cirrhinus mrigala*, Morphometric.

INTRODUCTION

The *C. mrigala* is grayish silver in colour with plain body, it has 12 to 15 dorsal fin rays, and 39 vertebrae in numbers (Kottelat, 2001). It can obtain maximum length upto 100cm (Robert, 1997) and the average commonly found individuals are of 40cm (Pethiyagoda, 1991) and maximum reported body weight have been 12.7kg (Talwar and Jhingran, 1991). In the subcontinent the *C. mrigala* is commonly known as mrigal, mori or morakhi and it is also recognized as Indian major carp due to their nomenclature and occurrence before the partition amongst the united Indian regions belongs to the ray finned fishes. This species is indigenous to the Indus River in Pakistan and Brahmaputra and Ganges Rivers, India. This fish forms one of the top candidates species of the sub-continent aquaculture due to its adaptability in the poly-culture, being herbivorous and harvested record of 463,520 tonnes in India in 2008 (FAO, 2010).

Silva (2003) conducted a study on the morphometric variation among sardine (*Sardina pilchardus*) populations from the northeastern Atlantic and the western Mediterranean. Their results indicated that there was also some evidence that fish from the western Mediterranean and the Azores form a separate morphometric group. These results question both the homogeneity within the Atlanto-Iberian sardine stock and the validity of its current boundaries. Rana *et al.* (2004) conducted a study with two hatchery and one river populations of *Labeo rohita* and *Catla catla* from different geographical locations in India. From their results they concluded that the *C. catla* population from Yamuna River Delhi, which is wild population, is highly diverse and is not included in any cluster. The Powerkheda population of *C. catla* placed in the same cluster consisting of *L. rohita* population as in the UPGMA tree. They postulated that mean diversity at population level is nearly 10% in *C. catla* and 1.33% at *L. rohita* level with 24 and 62%. Chauhan *et al.* (2007) conducted the study to investigate the different populations of *C. mrigala* from different Riverine sites in India. *C. mrigala* were collected from ten Riverine sites from the Indus, Ganges, Brahmaputra and Mahanadi basins. AMOVA analysis results showed that there is low differentiation among sub-populations. From these results it can be concluded that this low level of differentiation is may be due to the common ancestors of the populations in the pre-historic time and possible exchange of representatives between wild populations of *C. mrigala* in different River basins. Results of studies on *C. carpio* by Dayu *et al.* (2007) also indicated that there was a correlation between the clustering result and the geographical distribution. The clustering analysis by UPGMA, for the genetic diversity amongst the natural and hatchery raised populations of Indian major carp *C. catla* were studied. The 30-samples for each natural population were collected from the Halda, Jamuna and Padma Rivers and also from one hatchery population in Bangladesh. The geographic distances were directly correlated with the distances measured in gene diversity appeared in the dendrogram. Dendrogram showed 2-clusters, the population of River Halda appeared in one cluster other all natural populations including hatchery bunched in the second cluster. This indicates that first cluster from Halda was qualitatively different from the remaining populations (Rahman *et al.* 2009). El-Zaeem *et al.* (2012) conducted a study on the variation in phenotype based on morphometric character indices and meristic counts among different wild and cultured Nile tilapia (*Oreochromis niloticus*). The results revealed that there were significant differences ($P \leq 0.05$) in most of morphometric character indices and meristic counts among different wild and cultured Nile tilapia populations tested. The hierarchical cluster analysis based on each phenotype and genotype analysis grouped the four populations into two major category groups: Edku Lake, Manzalah Lake and Nile river populations group and cultured population group. Within these major grouping, wild Nile tilapia were grouped close together. Also, Edku Lake population appears to be more similar to that of Manzalah Lake population than that of Nile river population.

The intraspecific variation studies on *C. mrigala* species have no evidences in Pakistan and such work, which based on the morphometeric parameters for differentiation among the different varieties of mahaseer fish populations (Pervaiz *et al.*, 2012), intraspecific variability studies for plants have been conducted (Nisar *et al.*, 2010), and populations of *Heterodera zeae*, chickpea and walnut varieties (Abdollahi, 2009; Talebi *et al.*, 2008 and Asadian, 2005) in Iran, but the work on major carps or other aquatic animals is in small counts. The study "Clustering analysis for intraspecific variation studies amongst the populations of *C. mrigala*" was planned to sort out variations amongst the populations of same species collected from the different geographical locations due to environmental impacts or some other unknown factors.

MATERIALS AND METHODS

For this study the data about the morphometrics of 50-*C. mrigala* was collected from each site i.e. were the representatives of hatchery raised and natural populations of the Riverine systems of Punjab viz., UVAS-Fish Hatchery, C-block Ravi campus Pattoki District Kasur, Trimu Barrage at the junction of River Chenab and Jhelum near district Jhang, Taunsa Barrage at River Indus near Tehsil Kot Adu District Muzaffar Garh, Qadirabad Barrage at River Chenab near District Mandi Bahuddin and Baloki Barrage at River Ravi near Tehsil Bhai Phero District Kasur. Data regarding the morphogenetic parameters viz., body weight, fork length, total length and lengths of dorsal, caudal, anal, pectoral and pelvic fins of each individual were recorded. Ten representative samples collected from each geographical location and selected randomly from the total fifty samples of each site were used in clustering analysis. Agglomerative Hierarchical Clustering (AHC) was done by using Pearson Correlation Coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as Agglomeration method by XLSTAT 2012 version 1.02.

Table 1. Summary statistics

Variable	Minimum	Maximum	Mean	Std. deviation
Weight	204.000	315.000	258.480	27.921
Fork Length	20.600	27.600	25.288	1.431
Total Length	24.400	31.500	28.886	1.439
Dorsal Fin	3.200	5.100	4.238	0.423
Caudal Fin	3.100	5.200	4.072	0.512
Anal Fin	2.300	4.900	3.574	0.612
Pectoral Fin	2.100	3.900	3.196	0.497
Pelvic Fin	1.400	3.400	2.568	0.518

RESULTS AND DISCUSSION

Based on the most correlated morphometeric parameters of *C. mrigala* to develop correlation amongst the individuals of this species collected from the different geographical locations, a dendrogram was generated by clustering analysis (Figure 1).The dendrogram developed by this method by the presented data divided the populations of *C. mrigala* in to four major clusters or classes (Figure 2). The figure 1 also showed that except class 4 all the classes were further divided into sub classes. In the first class/cluster 26 individuals of same genetic structure or genotypes, in the second class/cluster 10 individuals of the same genotypes, in the third class/cluster 13 individuals with same genotypes and in the fourth class/cluster only one individual were grouped together. These results are in accordance with the findings of El-Zaeem *et al.* (2012), who conducted a study on the variation in phenotype based on morphometric character indices and meristic counts among different wild and cultured Nile tilapia (*O. niloticus*) and found that hierarchical cluster analysis based on each phenotype and genotype analysis grouped the four populations into two major category groups. As the results indicated that forth class consists of only one individual, these results are confirmation of the results postulated by Rana *et al.* (2004) who conducted a study with two hatchery and one river populations of *Labeo rohita* and *Catla catla* from different geographical locations in India. From their results they concluded that the *C. catla* population from Yamuna River Delhi, which is wild population, is highly diverse and is not included in any cluster.

The division of all the 50 *C. mrigala* samples collected from different geographical locations 10 from the each sampling sites was as follows in different four classes. Amongst the 26individuals of the same characters in the first class/cluster; the 8individuals were from the Hatchery samples, 7individuals were from the Indus River samples collected from Taunsa barrage, 4individuals included in this class/cluster were from the junction of Chenab and Jhelum Rivers at Trimu barrage, the 4samples were from the Chenab River collected from Qadirabad barrage while 3individuals in this first class/cluster were from the River Ravi sampled from Baloki barrage. From the 10individuals of the same characters in the second class/cluster; the 2individual was from the Hatchery samples, 2individuals were from the Indus River samples collected from Taunsa barrage, 2individual included in this class/cluster was from the junction of Chenab and Jhelum Rivers at Trimu barrage, in this class/cluster 2individual from the Chenab River collected from Qadirabad barrage while 2individuals in this first class/cluster were from the River Ravi sampled from Baloki barrage. The 13individuals of the same characters in the third class/cluster were consisted on the not any individuals from the Hatchery samples, 1individuals from the Indus River samples collected from Taunsa barrage, 4individuals from the junction of Chenab and Jhelum Rivers at Trimu barrage, the 4samples from the Chenab River collected from Qadirabad barrage while 4individualamongst the samples collected from the River Ravi sampled from Baloki barrage. In the fourth and last class/cluster there was only one individual having some unique characteristics and it was sampled from Ravi River from the Baloki barrage (Table 2).

Table 2. Results by class

Class	1	2	3	4
Objects	26	10	13	1
Sum of weights	26	10	13	1
Within-class variance	287.831	223.416	53.793	0.000
Minimum distance to centroid	0.636	1.610	1.203	0.000
Average distance to centroid	12.496	8.545	5.885	0.000
Maximum distance to centroid	49.118	40.928	14.484	0.000

Classes of *Cirrhinus mrigala*

ocIndividuals in Cluster/Class First:- Hatch.1, Hatch.2, Hatch.3, Hatch.4, Hatch.5, Hatch.6, Hatch.7, Hatch.9, Indus3, Indus4, Indus5, Indus6, Indus7, Indus9, Indus10, Trimu1, Trimu2, Trimu4, Trimu6, Qad.3, Qad.4, Qad.6, Qad.9, Ravi1, Ravi6, Ravi7

Individuals in Cluster/Class Second: - Hatch.8, Hatch.10, Indus1, Indus2, Trimu3, Trimu7, Qad.2, Qad.8, Ravi4, Ravi5

Individuals in Cluster/Class Third: - Indus8, Trimu5, Trimu8, Trimu9, Trimu10, Qad.1, Qad.5, Qad.7, Qad.10, Ravi3, Ravi8, Ravi9, Ravi10

Individuals in Cluster/Class Fourth:-Ravi2

The variance decomposition for the optimal classification values remained as, 27.28% for within class variation while 72.72% for the between class differences (Table 3). The class centroid for the each characteristics resemblance is also given (Table 4). The distance between the class/cluster centroids remained as; 50.820 for class one and two, 18.063 for class one and three, 14.564 for class one and four, 68.856 for class two and three, 36.708 for two and four while this distance between class three and four centroids was 32.408 (Table 5).The central objects of the four classes were sample 6 from the Hatchery for class first, sample1 from the Indus River from Taunsa barrage for the class second, sample 8 from the Indus River from Taunsa barrage for the third class and sample 2from the River Ravi from Baloki barrage for the fourth class/cluster (Table 6). In this clustering analysis the distances between the central objects of the different cluster/classes were also described (Table 7). These results are in accordance with the results of Chauhan *et al.* (2007) who studied different populations of wild *C. mrigal* from different River basins and concluded that there exist low level of differentiation between the populations of the same species and this may be due to common ancestry and exchange of individuals among the River basins. The results are also confirmation of the results indicated in the study conducted by Dayu *et al.*(2007) on the genetic similarity amongst the wild populations of *Cyprinus carpio* and concluded that there was a correlation between the clustering result and the geographical distribution.

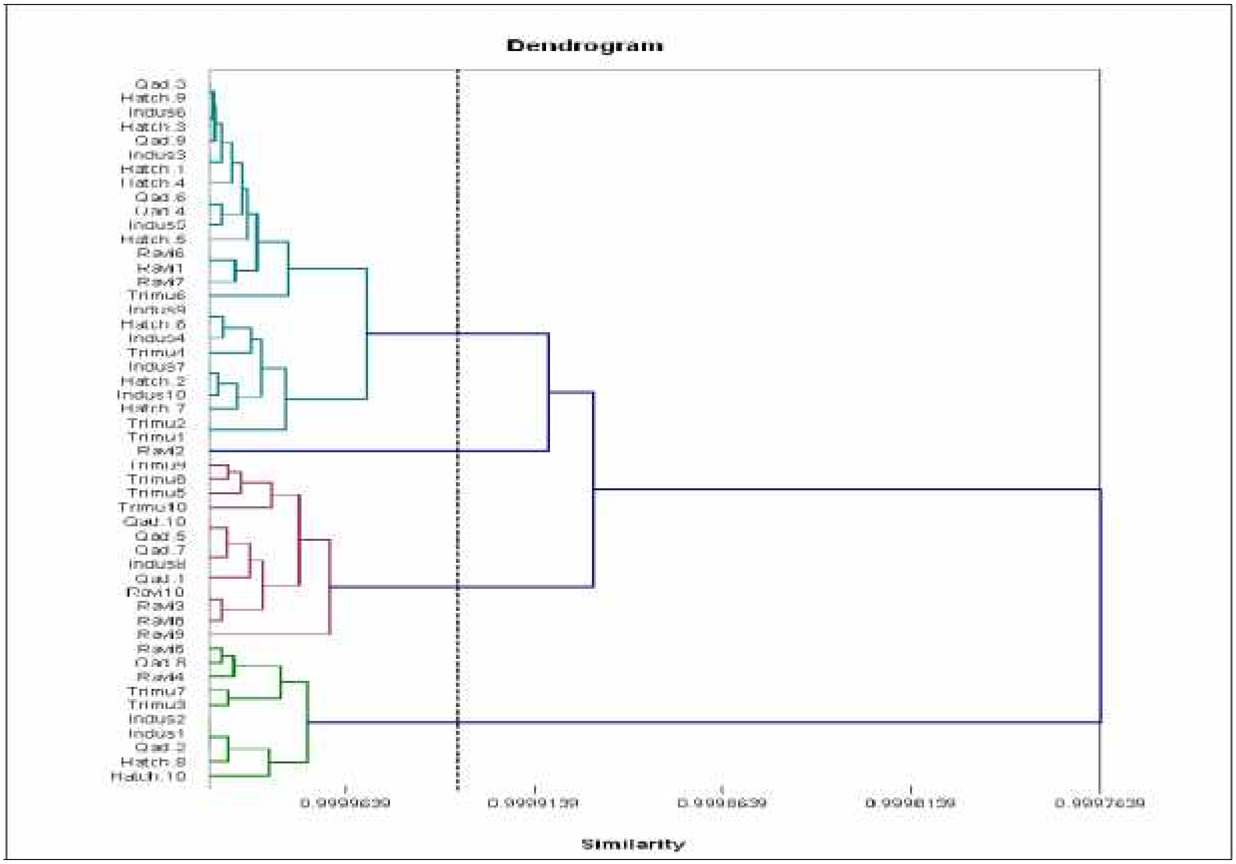

Figure 1: Dendrogram for Samples Distribution

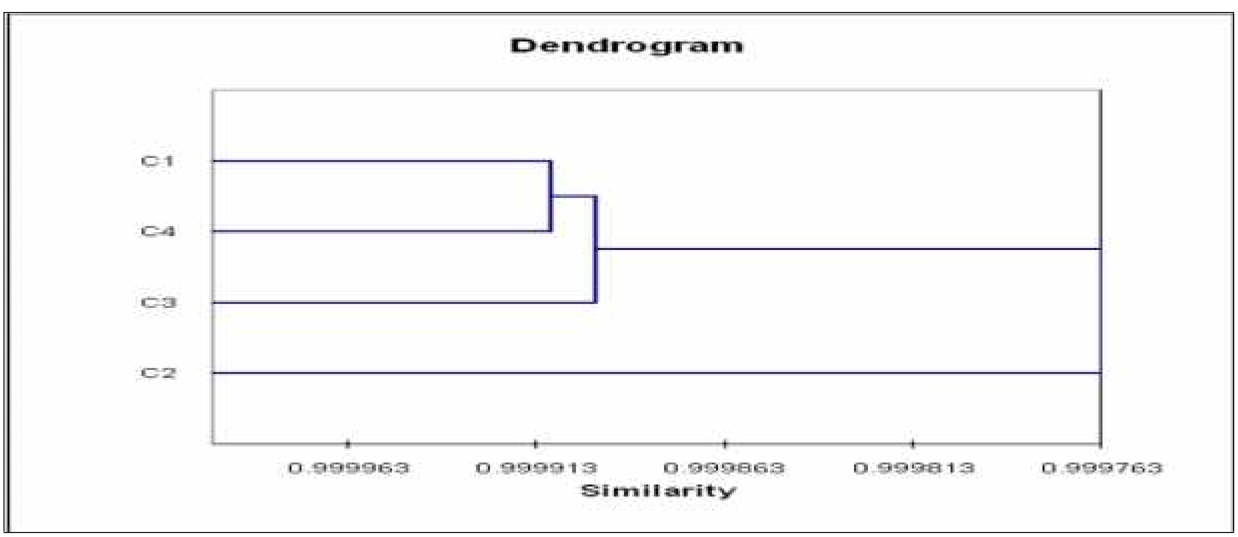

Figure 2: Dendrogram for Classes

Table 3. Variance decomposition for the optimal classification

	Absolute	Percent
Within-class	214.175	27.28%
Between-classes	570.876	72.72%
Total	785.051	100.00%

Table 4. Class centroids for the classes

Class	Weight	Fork Length	Total Length	Dorsal Fin	Caudal Fin	Anal Fin	Pectoral Fin	Pelvic Fin
1	252.731	24.885	28.623	4.223	4.150	3.554	3.292	2.631
2	303.500	26.420	30.100	4.660	4.490	3.970	3.470	3.020
3	234.692	25.115	28.638	3.946	3.615	3.362	2.854	2.146
4	267.000	26.700	26.800	4.200	3.800	2.900	2.400	1.900

Table 5. Distances between the class centroids

	1	2	3	4
1	0	50.820	18.063	14.564
2	50.820	0	68.856	36.708
3	18.063	68.856	0	32.408
4	14.564	36.708	32.408	0

Table 6. Central objects

Class	Weight	Fork Length	Total Length	Dorsal Fin	Caudal Fin	Anal Fin	Pectoral Fin	Pelvic Fin
1 (Hatch.6)	253.000	24.500	28.300	4.200	4.400	3.500	3.200	2.700
2 (Indus1)	305.000	26.500	30.400	4.700	4.800	4.200	3.600	3.300
3 (Indus8)	234.000	24.600	28.400	3.900	4.100	3.300	3.300	2.600
4 (Ravi2)	267.000	26.700	26.800	4.200	3.800	2.900	2.400	1.900

Table 7. Distances between the central objects

	1 (Hatch.6)	2 (Indus1)	3 (Indus8)	4 (Ravi2)
1 (Hatch.6)	0	52.094	19.007	14.321
2 (Indus1)	52.094	0	71.071	38.254
3 (Indus8)	19.007	71.071	0	33.130
4 (Ravi2)	14.321	38.254	33.130	0

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