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Allele Frequency Data of 4 Microsatellite Markers of *Cyprinus carpio* from Tapiza Hatchery in Albania

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Abstract – **The aim of this study is to characterize the carp population from a hatchery, based on microsatellite markers. Microsatellite markers are appropriate for evaluation of genetic diversity in animal populations. Thirty individuals were genotyped for four microsatellite markers, which were previously used also for the genetic characterization of other wild and hatchery carp populations. The individuals were selected from an important hatchery, that produce fingerlings for restocking of natural and artificial lakes of Albania. The results show a high level of genetic variability with mean observed and expected heterozygosity values of 0.483 and 0.835 respectively. All the markers were highly polymorphic, with a total of 66 alleles detected. PIC values for each marker were higher than 0.7 and displayed neutrality, proving to be appropriate for the genetic characterization of carp population. The population shows a high level of inbreeding of 43.5%. All markers deviated from HWE. The results provided here can help in the designing of a breeding program, for the carp which is an important fish species of our inland water.**

Keywords – **Genetic Variability, Hardy-Weinberg Equilibrium and Inbreeding Null Alleles.**

I. INTRODUCTION

Albania is an West Balkan country with large natural water resources, where aquaculture and fishery plays an important role for the country economy. In Albania, most lowland reservoirs are used for aquaculture [1]. A few hatcheries are involved in the cultivation of common carp (*Cyprinus carpio*), Chinese carps such as grass carp (*Ctenopharyngodon idellus*), bighead carp (*Hypophthalmichthys nobilis*), and silver carp (*Hypophthalmichthys molitrix*) for restocking purposes which are farmed in ponds (https: //www.eurofish.dk/albania). One of the largest hatchery in Albania, is Tapiza, which serve also, as experimental hatchery for Agricultural University of Tirana. A more detailed description of this hatchery is done previously by [2]

Carp fingerlings produced at this hatchery are used for restocking of lakes and reservoirs in Albania, therefore the genetic characterization is of great importance, since there are no information about genetic variation and structure of this stock. In this study we intend to study the genetic variation of carp population at Tapiza hatchery, by the use of 4 microsatellite markers. The same markers are used also previously to characterize carp populations from native lakes of Albania [3] [4].

II. MATERIAL AND METHODS

Fish fins were collected from individuals sampled from Tapiza hatchery as described by Bixheku et al., [2] Fragment length analysis of four microsatellite markers was carried out following the protocol described by Bixheku et al., [4].

Micro-checker [5], was used to test the possible presence of null alleles. Genalex software [6], was used to calculate observed and expected heterozygosity. The same software is used to calculate fixation index (F), in order to estimate within population genetic diversity. Ewens-Watterson test was performed by the use of POPGENE software [7], to test the neutrality for microsatellite markers.

Polymorphism information content (PIC), were calculated by Cervus software [8]. A Neighbor Joining (NJ) tree was built on the basis of Das distance matrix by using Populations v1.2.31 software [9]. The phylogenetic tree was visualized with TREEVIEW v1.6.6 [10].

In order to seek if the population has suffered a recent bottleneck effect the Bottleneck program was run [11]. Sign test, Standardized difference test and Wilcoxon test under the three models: Infinite Allele Model, IAM; Step Mutation Model, SMM and Two Phase Model, TPM were accomplished.

III. RESULT AND DISCUSSION

All four microsatellite markers used to characterize carp population of Tapiza hatchery were highly polymorphic (Table 1). The allelic frequencies for all loci are displayed in Figure 1. A total of 66 alleles were found for all loci. The number of alleles per locus ranged from 8 (MFW1) to 28 (MFW6), with a mean value of 16.5 ± 4.646, demonstrating a considerable amount of genetic diversity. The effective number of alleles varied from 3.983 to 19.882, with a mean value of 9.249 ± 3.744. This is an informative parameter, since it consider also the allelic frequencies. The effective number of alleles is much lower than the observed number of alleles. All the microsatellite markers have also high PIC values, ranging from 0.712 - 0.947, with an average of 0.878.

Table 1. Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Observed Heterozygosity (Ho), Expected (He) and Unbiased Expected Heterozygosity (uHe), and Fixation Index (F).

Locus	Na		Ne	I	Ho	He	uHe	F	PIC
MFW1	8.000		3.982	1.632	0.333	0.749	0.775	0.555	0.712
MFW6	28.000		19.882	3.158	0.808	0.950	0.968	0.150	0.947

Locus	Na		Ne	I	Ho	He	uHe	F	PIC
MFW7	10.000		4.017	1.738	0.364	0.751	0.768	0.516	0.721
MFW18	20.000		9.116	2.618	0.429	0.890	0.906	0.519	0.884
Mean	16.500		9.249	2.287	0.483	0.835	0.855	0.435	0.816
SE	4.646		3.744	0.365	0.110	0.051	0.050	0.095	

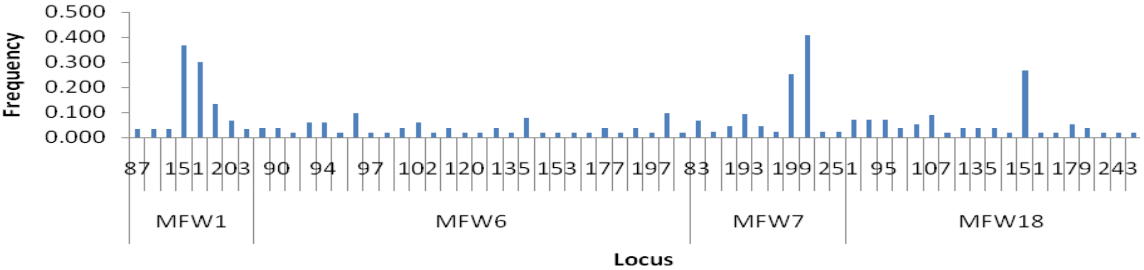

Fig. 1. Allele Frequencies by Tapiza hatchery population with graph over four microsatellite loci.

The observed heterozygosity had an average value over all loci of 0.483±0.110, meanwhile expected heterozygozity values ranged from 0.749 to0.950 with an average value of 0.835±0.051. High values of heterozygosity are in concordance with the polymorphism of used markers. All the markers displayed heterozygote deficit, as it is confirmed with high positive value of F. Shanon index varied from 1.632 to 3.158 with an average value of 2.287±0.365. Estimated values of fixation index (F) were all positive, indicating the heterozygotes deficiency in all loci and the presence of inbreeding in the population. The mean value of F index for all loci is 0.435±0.095, indicating a high level of inbreeding. The population was not in HWE for all the markers.

Ewens - Watterson test for neutrality of the markers shows that F-values for all markers lied between the lower and upper limit of 95% confidence region of expected F value (Table 2), that means that the markers are neutral.

Table 2. The Ewens-Watterson test for Neutrality at 4 microsatellite loci in Tapiza carp population.

Locus	k	Obs. F	SE*	L95*	U95*
MFW1	8	0.251	0.0042	0.1556	0.4067
MFW6	28	0.050	0.0001	0.0459	0.0777
MFW7	10	0.249	0.0045	0.0135	0.399
MFW18	20	0.110	0.0005	0.0702	0.154

The genetic relationship between carp individuals was illustrated by a NJ tree based on the DAS distance matrix (Figure 2).

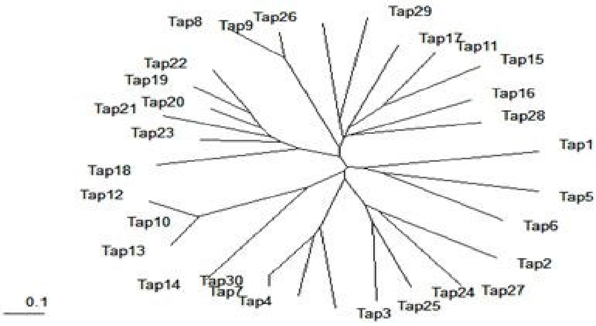

Fig. 2. Neighbor Joining (NJ) tree based on Das distance matrix

All tests carried out by Bottleneck software showed that the carp from Tapiza hatchery has not suffered a recent bottleneck. This is also supported by the typical L-shape graphic (Figure 3), where the alleles with the lowest frequencies (0.01–0.1) were found to be most abundant.

Carp population from Tapiza hatchery is characterized for the first time at the molecular level, using microsatellite markers. The results obtained show a high level of polymorphism for all the markers, displaying 8 or more alleles per locus and a total of 66 alleles. The lower number of effective alleles compared with the observed number of alleles, can be explained by the very low frequency of most of the alleles at each locus and therefore a very few alleles might have contributed to the major part of the allelic frequency at each locus [12]. Genetic markers having a higher number of alleles per locus and a higher degree of heterozygosity are considered as the most discriminatory for population and individual typing [13]. The polymorphism information content (PIC) value is a measure of the in formativeness of a genetic marker. According to Hilderbrand [14], the markers displaying PIC value greater than 0.7 is considered to be highly informative. Therefore all markers used in this study are highly informative and appropriate for the genetic characterization of carp population.

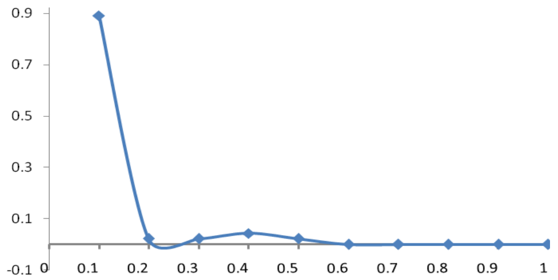

Fig. 3. Allele distribution by frequency ranges for the carp population.

Microsatellite markers displayed neutrality, supporting the use for the genetic analysis of carp population. The high average value of expected heterozygosity within population, can be explained with the large number of alleles per each locus [15]. Observed heterozygosity values were lower than expected, implying that the populations have lost some heterozygosity over the years [16]. Inbreeding is a major factor of causing reduction in heterozygosity in a population. The heterozygosity values are comparable with those of carp from Prespa Lake [17]. Danish and Singh [18] showed that the observed heterozygosity was 0.335 and the fixation index was 0.184 from a hatchery carp population

Positive values of fixation index per each marker, show a heterozygosity deficit, and the inbreeding is quite high at this population, with a mean value of 0.435.

All markers deviated from HWE. This can be explained by the presence of null alleles, which was also detected by micro-checker software, or due to high mutation rate and size homoplasy of microsatellite [19]. Differences between observed and expected heterozygosity, positve F values, and deviation from HWE indicate the inbreeding at the level of population.

Sultana et al., [20] consider inbreeding, lack of genetic characterization, presence of null alleles, as some of the main factors that decline the genetic diversity in hatcheries. NJ tree showed the resemblance between individuals. There is no recent bottleneck, showing that the minor alleles are intact in the population.

Knowledge of genetic variation and population structure is absolutely necessary for any efficient fish management and/ or conservation program [21]. The results of this study show that the carp population from Tapiza hatchery contains a high level of genetic diversity and provide new background of knowledge in population variability and breeding programs. Therefore, it is worthwhile to design breeding policies for these population, which is also used for the stocking of other lakes of Albania.

IV. CONCLUSION

Microsatellite markers used in this study are appropriate for the evaluation of genetic diversity of carp population. Population exhibited a high level of inbreeding, that can be explain by several reasons. The results provided here can help in the designing of a breeding program, since this population is also used for the restocking of natural and artificial lakes of Albania.

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