

RESEARCH ARTICLE

(Open Access)**Allelic frequencies of MFW7 microsatellite locus in carp (*Cyprinus carpio*) of two fish farming centers.**XHILIOLA BIXHEKU¹, ANILA HODA^{2*}, DHURATA BOZO¹¹Public Accreditation Agency for Higher Education²Agricultural University of Tirana, Albania

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Abstract

Common carp (*Cyprinus carpio* L) is among the most important freshwater fish species, distributed all over the world. We present preliminary data on genotyping individuals by DNA microsatellite marker. Allelic frequencies of one microsatellite locus was estimated on 60 individuals sampled randomly on Tapiza and Klos fish farming centers. Fragments were amplified by Polymerase Chain Reactions (PCR), and afterwards were separated on a 6% PAA denaturing gel. A total of 23 alleles were detected. Mean observed heterozygosity was 0.334 and expected heterozygosity was 0.816.

Keywords: observed and expected heterozygosity, marker, carp

Introduction

Common carp (*Cyprinus carpio* L) is an important domesticated freshwater fish worldwide. Carp from natural lakes of Albania, have been recently studied based on morphometric and meristic features [4] or microsatellite markers [1].

Microsatellites are tandem arrays of short nucleotide motifs, dispersed throughout eucaryotic nuclear genome and are characterized by the high level of polymorphism.

In the current study, we intend to characterize a microsatellite locus MFW7 from two hatcheries in Albania, Tapiza and Klos.

The carp hatchery of Tapiza is situated approximately 10 km from the Agricultural University of Tirana and include a total surface of 5 ha. This hatchery, the second largest carp hatchery in Albania, reproduces different carp species like common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Hypophthalmichthys nobilis*) and the Wuchang bream (*Megalobrama amblycephala*) and.

Generally, these carp species cannot reproduce in natural conditions in Albania and, hormonal induction in a specialized center like Tapiza hatchery, is necessary to stimulate reproduction.

The carp production company of Klos (Elbasan) is the largest carp hatchery in Albania and consists of 99 ponds of a total surface of 25 ha. This company produces annually 1.100.000 carp fingerlings of different species like common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Hypophthalmichthys nobilis*) and also different ornamental carp species like goldfish (*Carassius auratus*) and Koi carp (*Cyprinus carpio, haematopterus*).

Genetic variability of feral and hatchery stock of common carp are investigated in China [7] and Croatia [6].

The results displayed in our paper are preliminary data that will contribute to the genetic characterization of carp from these hatchery, which might be used for the carp breeding programmes in the future, since the fingerlings produced by them are

used for restocking reservoirs, lakes and other waterbodies in Albania.

Material and methods

A total of 60 individuals of common carp from two hatcheries of Tapiza and Klos were sampled and analyzed. Genomic DNA was isolated from fish fin by proteinase K digestion and salt out procedure. The PCR reaction for amplifying microsatellite locus MFW 7 was performed by the use of 2 mM dNTP mix 1U Taq polymerase, 50 mM MgCl₂, 10 ng primers, 50-100 ng DNA plus PCR buffer and bidest water up to final volume of 12.5 μ l. PCR amplification conditions were as follows: one preliminary denaturation step at 94°C (5 min), followed by strand denaturation at 94°C (45 sec), annealing at 56°C (45 sec) and primer extension at 72°C (1 min) repeated for 33 cycles and a final extension at 72°C(10 min).

Indicators as allelic frequencies, observed and expected heterozygosity estimates, observed number of alleles, effective number of alleles, departures from Hardy-Weinberg equilibrium were computed using Genalex software [3].

Results and discussion

The allele and genotype frequencies of MFW7 locus were determined in 2 populations of Tapiza and Klos hatchery.

In figure 1 are given the alleles and allelic frequencies for both population. The most frequent allele for Klos population was allele 99 and for Tapiza the allele 201. In table 1 are shown genetic diversity indices. There were identified 9 alleles at Tapiza and 20 at Klos population,

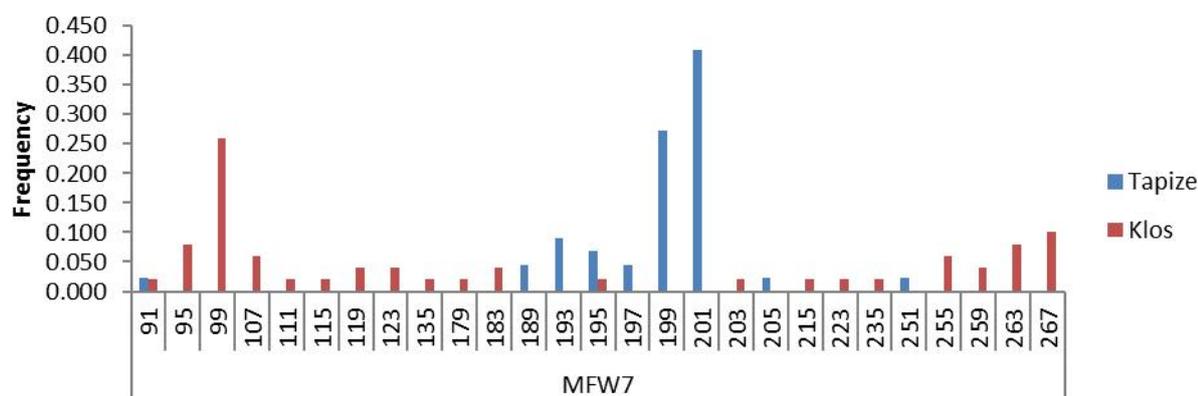


Figure 1: Allele Frequencies by Population with Graph over Loci

Table 1: Summary statistics of allelic distribution of MFW7 locus in both populations

| Pop | N | Na | Ne | I | Ho | He | uHe | F |
|--------|--------|--------|-------|-------|-------|-------|-------|-------|
| Tapiza | 22 | 9.000 | 3.841 | 1.660 | 0.227 | 0.740 | 0.757 | 0.693 |
| Klos | 25 | 20.000 | 9.259 | 2.620 | 0.440 | 0.892 | 0.910 | 0.507 |
| Total | 23.500 | 14.500 | 6.550 | 2.140 | 0.334 | 0.816 | 0.834 | 0.600 |

N: Sample Size, Na: No. Alleles, Ne: No. Effective Alleles, I: Information Index, Ho: Observed Heterozygosity, He: Expected F: Fixation Index

Gene diversity was 0.816 for the whole population ranging from 0.740 (Tapiza) to 0.892 (Klosi). These results confirm that the microsatellite analyzed here is useful marker. In both populations is

observed a high rate of inbreeding, with an average over the whole population of 0.60.

In a previous study, of *Cyprinus carpio* from three natural lakes of Albania [1], were observe a total

of 48 alleles, and the gene diversity was 0.956. Tomiljanovic et al., 2013 identified 14 alleles for this marker in fish populations from Croatia. Thai et al [5] observed 10 alleles for this marker for the common carp of Vietnam, with a gene diversity of 0.68 and with an inbreeding value of 0.22. This marker is analyzed in Gomishan bay and Gorganroud River [2] where 20 and 18 alleles were detected respectively, with a gene diversity higher than 0.9.

The results displayed in this study have to be considered as preliminary data and first efforts to estimate the genetic pool of carp from two important fish farms that are used to produce fingerlings for restocking of natural carp populations in Albania. The results, along with other studies in our country in the field, can be used in the future for establishing breeding programs of *Cyprinus carpio* from natural lakes of Albania

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