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Genetic diversity of *Cyprinus carpio* from Ohrid Lake, estimated by four microsatellite loci

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Abstract

Common carp (*Cyprinus carpio* L) is an important fish species of Ohrid lake. The aim of the present study was to evaluate the genetic diversity of common carp using microsatellite markers. A total of 30 individuals were genotyped for 4 microsatellite loci (MFW1, MFW6, MFW7, MFW18). All the microsatellite loci were polymorphic. A total of 84 alleles were distinguished. The allelic number varied from 19 to 23 with mean allelic number of 21. The effective number of alleles varied from 9.92 to 11.84 with a mean of 11.66. The observed heterozygosity ranged from 0.32 to 0.73 with mean value of 0.57. The loci were highly informative. The PIC values ranged from 0.89 to 0.91 with mean of 0.90. The values of Shannon information index (I) ranges from 2.63 to 2.78. The population was not in Hardy-Weinberg equilibrium (HWE) for all of four loci. The mean expected heterosygosity value (0.91) was higher the mean observed heterosygosity (0.57). The mean fixation index (F) over the loci was 0.37. A bottleneck analysis is carried out, which shows no recent bottleneck. Factorial Correspondence Analysis (FCA) shows a close relationship between individuals.

Key words: common carp, microsatellite, heterosygosity, genetic diversity, polymorphic.

Introduction

Common carp (*Cyprinus carpio*) is among the most important freshwater species of Albanian Natural Lakes. Originating from the branches of river Danube it is considered a native species of our Lakes [18; 8; 10]. It lives in different water conditions not only in Albania but all over the world [2]. This is due to some features attributed to this species such as: high potential to adapt with environmental conditions, rapid growth, early maturity and high reproductive capability, the ability to live in different water quality and flexibility with the diet food [6, 7]. The qualities attributed to common carp have affected his morphological features. Several studies on morphological features, the capability of several phenotypes of common carp to adapt with the environmental conditions of Albanian Natural Lakes are done [19, 15, 11, 20, 12].

Recently, molecular markers are used in order to estimate genetic differences within and between carp populations. Microsatellites are considered as marker of choice and are widely used to measure genetic diversity of *Cyrpinus carpio* populations worldwide [21; 9; 13; 24, 22; 1]

In Albania there are not similar studies carried out before. In the present study, we intended to evaluate genetic diversity of *Cyprinus carpio* from Ohrid Lake using four microsatellite markers.

Material and methods

Fish fin were collected from 30 individuasl of Ohrid Lake and were preserved in 97% (v/v) ethanol. Genomic DNA was isolated by proteinase K digestion, chloroform extraction and izopropanol precipitation. A total of four microsatellites (MFW1, MFW6, MFW7, MFW18) were used in this study. PCR was performed with conditions comprising 1×PCR buffer, 0.2mM dNTP, 2.5µM primers, 1.5mM $MgCl_2$, 1U *Taq* DNA polymerase. Forward primer was IRDye 700 labeled. Thermal cycling conditions were: 8 denaturing cycles in 94°C for 30 sec, annealing in 62°C for 30 sec; and 72°C for 1 min; followed by 26 cycles, with denaturing temperature at 57°C with a final extention at 72°C for 10 minutes. PCR products were separated in 6% denaturing polyacrylamide gel using *4300 DNA Analyser*. Fragment length of PCR products was determined relative to 50-350 size standards (LICOR).

Number of alleles per locus, observed heterozygosity (Ho) and expected heterozygosity (He) were calculated using Genalex 6.5 program [16]. The same program was used to test departures from Hardy Weinberg equilibrium. Polymorphic information content (PIC) was estimated using the Cervus software [14]. To detect whether the carp population has experienced a recent bottleneck, an analysis was carried out, using Bottleneck software [17]. Mode shift indicator, which discriminates bottlenecked populations from stable populations, was used. Factorial Correspondence Analysis (FCA) was carried out using GENETIX v4.02 [3].

Results and Discussions

The estimated values of different measures of genetic variation in *C. carpio* from Ohrid Lake, across 4 microsatellite loci are presented in Table 1.

Table 1. Measures of genetic variation in common carp

Locus	*N*	*Na*	*Ne*	*I*	*Ho*	*He*	*UHe*	*Sign*	F_{IS}	*PIC*
MFW1	30	23.0	11.84	2.75	0.63	0.91	0.93	* * *	0.308	0.910
MFW6	26	19.0	12.51	2.71	0.73	0.92	0.93	* *	0.206	0.915
MFW7	25	22.0	12.37	2.79	0.60	0.91	0.93	* * *	0.347	0.914
MFW18	25	20.0	9.92	2.63	0.32	0.89	0.91	* * *	0.644	0.892
Mean	26.500	21.0	11.66	2.72	0.57	0.914	0.931		0.376	0.907
SE	1.190	0.91	0.59	0.03	0.08	0.005	0.005		0.094	

Number of individuals (N), number of alleles (Na), Effective number of alleles (Ne) Information index (I), Observed heterozygosity (Ho), Expected heterozygosity (He), Unbiased heterozygosity (UHe), Fixation index (F),Polymorphism information content (PIC).

ns=not significant, ** P<0.01, *** P<0.001

Number of alleles varied from 19 (MFW6) to 23 (MFW1 There were identified 58 alleles for 4 microsatellite markers. Number of effective alleles varied from 9.92 (MFW18) to 12.51 (MFW6). Gene diversity was 0.57, lower than five wild Central Asian common carp analyzed for four microsatellite loci [0.851; 9], twenty Vietnamese wild common carp analyzed for four microsatellite loci [0.77-0.83; 22]. The same gene diversity (0.57) has published Li et al. 2013 [13] who analyzed thirty microsatellite loci for six wild populations of *Cyprinus carpio* of China. Our value was greater than ten Croatian common carp analyzed for fifteen microsatellite loci [0.369-0.612; 23]. Observed heterozygosity ranged from 0.32 (MFW18) to 0.73 (MFW6). The values of expected heterozygosity ranged from 0.89 (MFW18) to 0.92 (MFW6). The mean value of observed heterozygozity (0.57) was lower than the mean expected heterozygosity (0.914), indicating losses in heterozygosity values. All loci showed significant deviation from Hardy Weinberg equilibrium (P<0.01, P<0.001). F_{IS} values for all markers ranged from 0.206 (MFW6) to 0.644 (MFW18), with an average value of 0.376 indicating high level of heterozygote deficit. This may be due to the inbreeding effect that may have occurred to common carp of Ohrid Lake. Reduction in heterozygosity values were shown in previous results for Ohrid and Shkodra Lake [4]. Similar positive F_{IS} values are observed for 10 Croatian populations of Common carp, using 15 microsatellite markers [23].

The value of Shannon information index (I) ranged from 2.63 to 2.79. The PIC values ranged from 0.892 (MFW18) to 0.915(MFW6). Mean PIC value is 0.90. A gene is highly informative if PIC value is higher than 0.5 [5]. All of four our loci were highly informative.

We used Wilcoxon sign rank test under SMM, IAM and TPM evolution models for estimating a recent bottleneck, because, of its relatively high statistical power and it can be used with as few as four polymorphic loci and any number of individuals. The heterozygosity was not significant under all models (p > 0.05). The mode-shift indicator showed a normal L-shaped, indicating lack of population bottleneck (Figure 1). The most abundant alleles are those with low frequency (0.01 – 0.1).

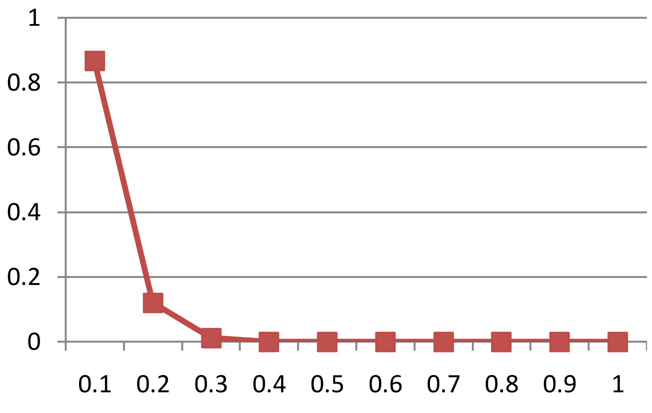

Figure 1: Mode shift curve showing lack of recent bottleneck in common carp

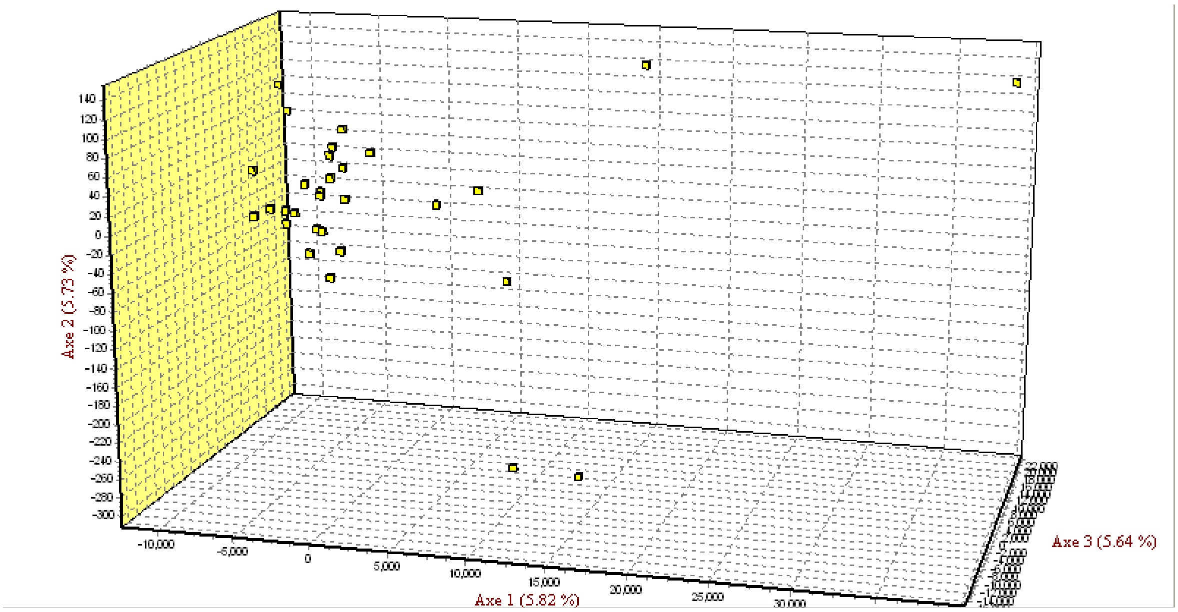

Figure 2: Factorial Correspondence Analysis (FCA) between individuals of common carp

Factorial correspondence analysis (FCA) suggests that first axis accounted for 5.82%; second 5.72% and third 5.64% of the total variance (Figure 2) showing a close genetic relationship between individuals. A few individuals show higher genetic distance from the rest of the populations, and thus greater genetic diversity.

The whole population showed a high deficit of heterozygotes of 37.6% that supports also a close genetic relationship between sampled individuals displayed by FCA.

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