

## GENETIC DIVERSITY OF *CYPRINUS CARPIO* OF PRESPA LAKE BASED ON MICROSATELLITE LOCI

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### Përmbledhje

Krapi (*Cyprinus carpio*) i Liqenit të Prespës është ndër speciet e peshqëve më të rëndësishëm dhe me sasi më të madhe në këtë liqen. Pesë lokuse mikrosatelitë u përdorën për të vlerësuar diverzitetin gjenetik të krapit. Për këtë qëllim u gjenotipizuan gjithsej 30 individë. Të gjithë markerët e përdorur në këtë punim ishin polimorfikë, ata paraqiteshin me vlerë mesatare të PIC prej 0.836. Numri i përgjithshëm i aleleve për të pesë lokuset ishte 45. Vlera mesatare e heterozigotisë së vëzhguar  $H_O = 0.345$  ishte më e ulët se vlera mesatare e heterozigotisë së pritur  $H_E = 0.847$ . Popullata nuk ishte në ekuilibër gjenetik për të pesë lokuset. Vlerat e indeksit të informacionit Shanon (I) varionin nga 1.65-2.69 ndërsa Indeksi mesatar i fiksimit ishte 0.588. Në popullatë nuk vërehej fenomeni grykë-shishe.

### Abstract

*Cyprinus carpio* of Prespa Lake is one of the most important and abundant fishes of the lake. Five microsatellite loci were used to evaluate its genetic diversity. For this reason a total of 30 individuals were genotyped. All the markers were highly polymorphic, mean PIC value was 0.836. The total number of alleles was 45. Mean observed heterozygosity  $H_O = 0.345$  was lower than mean expected heterozygosity  $H_E = 0.847$ . The population was in Hardy Weinberg equilibrium for none of the loci. On the other side, the values of Shanon information index (I) ranged from 1.65 -2.69, while the mean fixation index over the five loci was 0.588. The bottleneck analyses showed no recent bottleneck.

**Key words:** Common carp, microsatellite, allele, expected heterozygosity, observed heterozygosity.

### Introduction

Prespa Lake is located at the south-east part of Albania. At an altitude 853 m (above sea level) it is the highest tectonic Lake in the Balkan Peninsula (UNDP-GEF, 20012). Prespa Lake is shared between Albania, Former Yugoslavian Republic of Macedonia and Greece (FAO, 2005).

Albanian part of Prespa area in 1999 was designed as Prespa National Park (PNP) not only due to the specific geographical features but also for its very high biodiversity, extremely rich flora and fauna (Grazhdani, 2010).

There are nineteen fish species in the Prespa Lakes (UNDP- GEF, 2012-2016). Bleak, carp and barbell are the most important and abundant fishes in the Lake (MMAUJ; Kapedani & Hoxha, 2007; Flloko, 2005). Common carp represents a stable population in the lake. Fishing of carp, as one of the most abundant fish species in the lake, is an important source of income for the local community (Grazhdani, 2010).

It is reported that in Prespa Lakes are present illegal fishing activities, even during the period from April to October. This phenomenon has caused the deterioration of fish production and the presence of small caught common carp fishes (Grazhdani et al., 2010; Antonis & Zacharoula, 2009).

Another risk for carp population is the phenomenon of eutrophication and changes in water volume. However, to keep a stable population, fingerlings from Zvezda hatchery have been introduced into Prespa lake (Kapedani & Hoxha, 2007).

Studies on worm water fishes have listed common carp as an important and abundant fish for the prespa Lake (Rrakaj, 1995; Flloko, 2005; Shumka et al., 2008).

Comparative studies on the basis of index exteriority and growth factor of carp in Prespa Lake and other natural lakes and growth economies were carried out in 1998. They showed that Prespa Lake carp had the worst growth indicators (Spaho *et al.*, 1998). Morphometric and meristic features of Prespa Lake carp revealed smaller body size in comparison with carp of Belshi Lake (Shermadhi & Spaho, 2013).

The aim of the present study was to evaluate the genetic diversity of *Cyprinus carpio* of Prespa Lake by microsatellite markers.



**Figure 1.** Prespa Lake**Materials and methods**

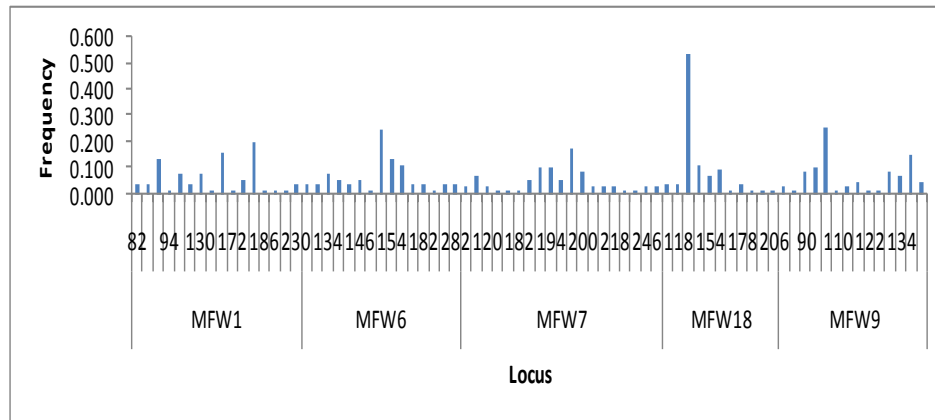
Fish fins were taken from 30 individuals captured in the Albanian part of Great Prespa Lake during August-September 2013; they were used for the DNA isolation according to standard procedures. Five microsatellite loci were used in the study (MFW1, MFW6, MFW7, MFW18, MFW9). PCR was performed with conditions comprising 1×PCR buffer, 0.2mM dNTP, 2.5µM primers, 1.5mM MgCl<sub>2</sub>, 1U Taq DNA polymerase. Forward primer was IRDye 700 labeled. PCR was performed in a total of 33 cycles with denaturing cycles in 94°C for 30 sec, annealing for 30 sec according to the T<sub>m</sub> and instructions specified at the LICOR manual and extension at 72°C for 1 min.

The amplified fragments were separated by vertical electrophoresis in 6% denaturing polyacrylamide gel using 4300 DNA Analyser (LICOR). Average expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosity values were calculated by Genalex 6.5 program (Smouse & Peakall, 2003). The same software was used to evaluate Hardy- Weinberg Equilibrium. A polymorphism information Content (PIC) value, that is a statistical assessment of informativeness of a marker, was estimated by Cervus software (Marshall, 1998). Factorial Correspondence Analysis (FCA) was carried out by Genetix software v4.02 (Belkhir K et al., 2001). Bottleneck software analyses were used to detect whether carp population of Shkodra Lake has experienced a recent bottleneck, for this reason we applied Wilcoxon sign-rank test (Piry S et al., 1999). The above test has high power and can be used as few as four polymorphic loci. Mode shift indicator, which discriminates bottlenecked populations from stable populations, was used as well.

**Results and discussions**

Total number of alleles over the five loci was 45. MFW7 locus presented the highest number of alleles (19 alleles) while MFW18 locus had the lowest number of 11 alleles. Over all the loci the allele 146 had the highest frequency 0.613 and the allele 172 and 226 had the lowest frequency 0.02.

Allelic frequencies are displayed in Figure 2.



**Figure 2.** Allele frequencies of five microsatellite loci

Genetic diversity parameters are shown in table 1. All markers were highly polymorphic. Mean PIC value was 0.836, therefore these markers appeared to be highly informative (Botstein *et al.*, 1980) and appropriate in genetic diversity study. Gene diversity was 0.345 that is lower than gene diversity of Ohrid Lake (Biba *et al.*, 2014). All markers displayed significant departure from Hardy Weinberg equilibrium. Departures from HWE were also published for common carp of Asia, Central Asia and East/ South East Asia (Kohlman *et al.*, 2003), Vietnamese carp population (Thai *et al.*, 2007), Southern Caspian carp (Yousifian & Laolei, 2011). Observed heterozygosity ( $H_O$ ) calculated in the present investigation ranged from 0.160 (MFW1) to 0.483 (MFW9) with a mean value of 0.345. Expected heterozygosity ( $H_E$ ) ranged from 0.680 (MFW18) to 0.916.

Mean value of expected heterozygosity 0.847 shows the variation in common carp of Prespa Lake. Mean observed ( $H_O$ ) value was lower than mean expected heterozygosity ( $H_E$ ) values, indicating decrease of heterozygosity values. Our values for observed and expected heterozygosity were lower than the values published by Lehoczky *et al.*, 2005 ( $H_O = 0.557$ ,  $H_E = 0.70$ ) for six strains of Hungarian common carp that were analyzed for 12 microsatellite loci. In addition, analysis that have been carried out on Bangladesh common carp revealed an observed heterozygosity of 0.54-0.67, and expected heterozygosity of 0.60-0.73 in three captive population analyzed for 3 microsatellite markers (Alam & Alam, 2012), they were lower than 10 Croatian common carp populations analyzed for 15 microsatellites ( $H_O: 0.0369-0.612$ ,  $H_E: 0.654-0.736$ ) (Tomljanovici *et al.*, 2013). Inbreeding values ( $F_{IS}$ ) ranged from 0.445 (MFW9) to 0.820 (MFW1), with a mean value of 0.588 indicating high level of heterozygote deficiency.

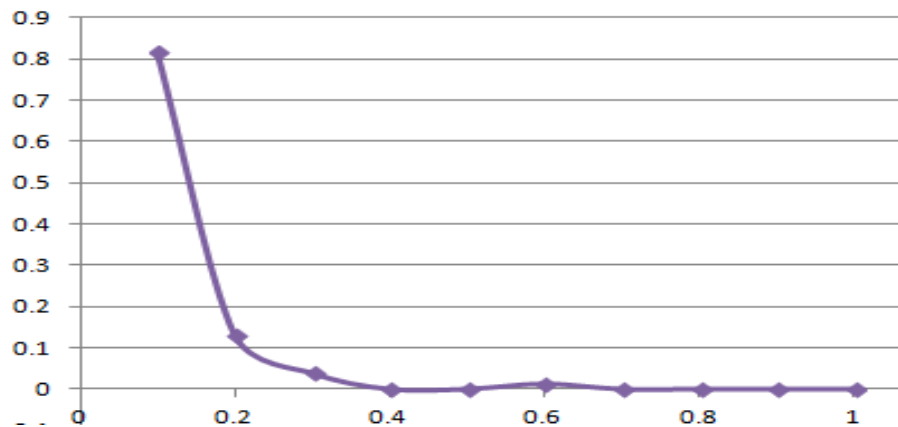
**Table 1.** Genetic parameters in common carp

	MFW1	MFW6	MFW7	MFW9	MFW18	Mean	SE
<b>N</b>	25	26	28	27	29	27.000	0.707
<b>Na</b>	16	15	19	11	14	15.000	1.304
<b>Ne</b>	9.058	8.503	11.879	3.122	7.680	8.048	1.420
<b>I</b>	2.448	2.421	2.697	1.653	2.299	2.304	0.175
<b>H<sub>O</sub></b>	0.160	0.462	0.286	0.333	0.483	0.345	0.059
<b>H<sub>E</sub></b>	0.890	0.882	0.916	0.680	0.870	0.847	0.043
<b>F<sub>IS</sub></b>	0.820	0.477	0.688	0.510	0.445	0.588	0.072
<b>PIC</b>	0.880	0.873	0.910	0.660	0.858	0.836	
<b>Sign</b>	***	***	***	***	***		

Number of individuals (N), number of alleles (Na), Effective number of alleles (Ne) Information index (I), Observed heterozygosity (H<sub>O</sub>), Expected heterozygosity (H<sub>E</sub>), Fixation index (F<sub>IS</sub>), Polymorphism information content (PIC).

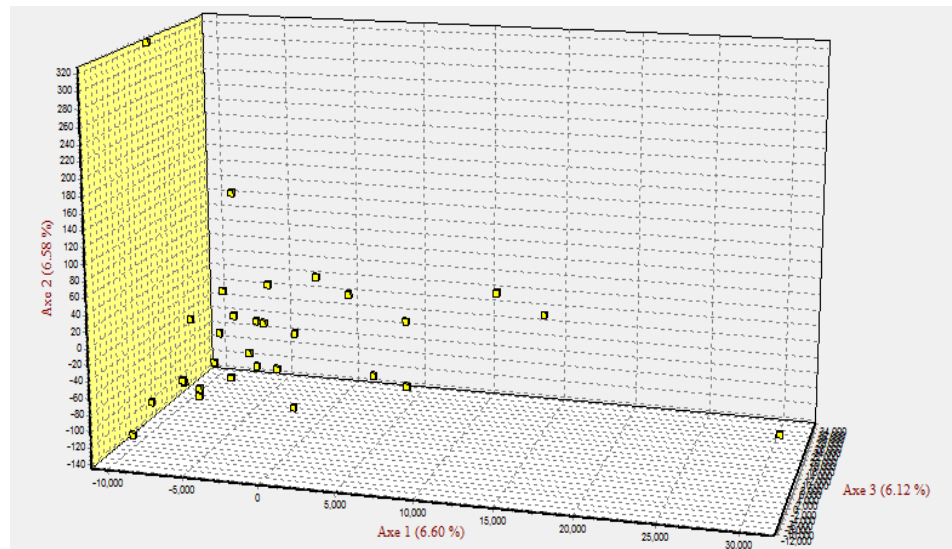
The value of Shanon information Index (I) ranged from 1.65 -2.69.

Wilcoxon test was developed under SMM, IAM and TPM evolution models. Under all the three models was accepted the null hypothesis of mutation drift equilibrium ( $p > 0.01$ ). The mode shift indicator test was also utilized as a second method to detect potential bottleneck. The proportions of alleles showed a normal L shaped distribution. The alleles with low frequencies (0.01-0.1) are the most numerous, supporting also the result of Wilcoxon test (Figure 3).



**Figure 3.** Mode shift curve showing lack of recent bottleneck in common carp

Factorial correspondence analyses suggests that first axis accounted for 6.60%, the second axis for 6.58% and the third axis for 6.12% of the total variance (Figure 4) showing close genetic relationship between individuals. Only a few individuals show higher genetic distance and higher genetic diversity compared to the other part of the population.



**Figure 4.** Factorial Correspondence Analyses (FCA) between individuals of common carp

The whole population showed a high deficit of heterozygotes of 58.8%, which supports also a close genetic relationship between sampled individuals displayed by FCA. The deficit in heterozygosity is higher than that observed for Ohrid Lake carp (37.6%) (Biba *et al.*, 2014).

### Conclusion

- All the markers used in the study were highly polymorphic.
- All the loci showed decrease of heterozygosity values and departure from Hardy-Weingerg equilibrium.
- No recent bottleneck was detected for *Cyprinus carpio* of Prespa Lake

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