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# PRELIMINARY RESULTS OF MOLECULAR GENETIC INSPECTION OF HYPOPHTHALMICHTHYS GENUS IN LAKE BALATON, HUNGARY

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## Abstract

The planktivorous filter-feeding Asian carp or bigheaded carp (bighead carp *Hypophthalmichthys nobilis*, silver carp *H. molitrix* and their hybrids) had been introduced to Hungary. Artificial hybridisation and stocking to Lake Balaton was systematic in the 70's. Though the stocking of the lake was stopped and banned in 1983, the biomass of bigheaded carp is still abundant in the lake (despite several preceding studies suggesting that *Hypophthalmichthys spp.* are not able to reproduce in this lacustrine environment).

The objective of the present analysis was to evaluate the genetic status of filter-feeding Asian carps living in Lake Balaton by using molecular genetic markers. Ten autosomal microsatellite markers and the mitochondrial cytochrome oxidase I gene were used for the population genetic analyses and for the determination of the proportion of hybrid individuals. The fish stock of Lake Balaton was compared to silver carp and bighead carp individuals originating from the live gene bank in Parbatipur, Bangladesh. The results showed that the Balaton stock of filter feeding Asian carps is genetically variable with a high number of observed alleles at each loci with an average of 14. The results of the microsatellite analyses, complemented by the mitochondrial maternal line identification, clearly showed the hybrid status of the Lake Balaton *Hypophthalmichthys spp.* stock. The microsatellite analyses also showed the lack of Hardy-Weinberg equilibrium in the stock suggesting that the individuals are not forming a natural reproductive population.

Keywords: filter feeding Asian carps, hybridization, microsatellite markers, cytochrome oxidase I

## Introduction

The planktivorous filter-feeding Asian carp (bighead carp -*Hypophthalmichthys nobilis*, silver carp -*H. molitrix* and their hybrids) had been introduced into more than 30 countries around the world, including Hungary and Lake Balaton. Although the stocking of *Hypophthalmichthys spp.* in the lake was banned in 1983 by the government, they are still abundant in the lake (despite several preceding studies suggesting that *Hypophthalmichthys spp.* are not able to reproduce in this lacustrine environment). The present study is part of a larger program analysing the ecological impacts and life history of *Hypophthalmichthys spp.* in Lake Balaton, including their adverse effects on water quality and native fish populations, the rate of hybridization, growth parameters and feeding habits. The objective of the present analysis was to evaluate the genetic

status of filter-feeding Asian carps living in Lake Balaton by using molecular genetic markers for population genetic analyses and the determination of the proportion of hybrid individuals.

## Materials and methods

Fin clip samples were collected from 110 *Hypophthalmichthys* spp. individuals captured in Lake Balaton and from 25 silver carp and 24 bighead carp individuals originating from the live gene bank in Parbatipur, Bangladesh (Mia et al. 2005). DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the protocol of the manufacturer. Ten autosomal microsatellite markers (Hmo01, Hmo02, Hmo03, Hmo13, Hmo33, Hmo34, Hmo36, Hmo37, Hmo39 and Hmo40) were used for the genetic analysis of the individuals as described in Gheyas et al. (2006) with some modifications (primers were fluorescently labeled with FAM, TET and HEX dyes and the length of different microsatellite alleles were assessed by running the multiplexed PCR products against GeneScan™ –500 LIZ® (Applied Biosystems, Foster City, California) size standard on a 3130 Genetic Analyzer. Alleles were visualized using GeneMapper version 4.0 software (Applied Biosystems, Foster City, California). The analysis of mitochondrial cytochrome oxidase I gene was used to identify the maternal lineage of individuals. The polymerase chain reactions were carried out as described by Ivanova et al. (2007) with COI-F (5'-TTCTCCACCAACCACAARGAYA TYGG-3') and COI-R (5'-CACCTCAGGGTGTCCGAARAAYCARAA-3') primers. The sequences were determined by BigDye 3.1 sequencing Kit and 3130 Genetic Analyzer (Applied Biosystems, Foster City, California). For data analyses GenAIEx V. 6.5, MEGA7 and DnaSP v5 softwares were used.

## Results and discussion

The number of microsatellite alleles was found between 4 and 20 with an average of 14 in the Lake Balaton population, 9.3 in silver carp and 9.1 in bighead carp, respectively. Expected heterozygosity values of Lake Balaton stock ranged from 0.59 to 0.91 on different loci within the collected sample group with an average of 0.77, while observed heterozygosity ranged from 0.35 to 0.87 with an average value of 0.68. The expected and observed values differed significantly in the case of all markers, indicating that the stock is not in Hardy-Weinberg equilibrium. The stock of Lake Balaton and the bighead carp population showed the signs of light inbreeding. The genetic distances between the populations were between 0.35 and 0.69. The UPGMA dendrogram based on Nei's  $D_a$  distance data showed that the stock of Lake Balaton is between the two gene bank stocks, closer to silver carp. The assignment test (Bayesian method, Rannala and Mountain 1997) and the Structure analysis (Pritchard et al. 2000) revealed that all individuals from Lake Balaton are hybrids with a higher proportion of silver carp genome; 86% of the individuals carried silver carp mitochondrial sequences, while only 14% of them had bighead carp maternal line. The numbers of the identified haplotypes were 38 and 8, respectively.

The results of the microsatellite analyses, complemented by the mitochondrial DNA based maternal line identification, clearly showed the hybrid status of the Lake Balaton *Hypophthalmichthys* spp. stock. Many times there were contradictions between the maternal line typing and the microsatellite based genome distributions, indicating a higher level of hybridization within the stock. The microsatellite analyses showed the lack of Hardy-Weinberg

equilibrium in the stock suggesting that the individuals are not forming a natural reproductive community. Although the direct stocking of Asian filter feeding carps in Lake Balaton has been prohibited since 1983, the individuals in the stock are typically younger than 33 years old. The recruitment of the stock most probably originates from the artificial hybrid production of the aquaculture companies in the lake's water basin (Boros et al., 2014, Vitál et al. 2015). This hypothesis is confirmed by the microsatellite analyses and the high variance of the mitochondrial haplotypes as well. However, the occasional natural reproduction of the introduced Asian carps cannot be ruled out.

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