

## GENETIC VARIABILITY OF AN INVASIVE FRESHWATER FISH, GIBEL CARP *CARASSIUS GIBELIO* (BLOCH, 1782) IN TURKEY REVEALED BY SEQUENCES OF MITOCHONDRIAL CYTOCHROME OXIDASE I GENE

SEVAN AĞDAMAR<sup>1</sup>, ALI SERHAN TARKAN<sup>1</sup>

<sup>1</sup>*Muğla Sıtkı Koçman University, Faculty of Fisheries, 48000, Muğla, Turkey*

GENETSKE VARIJACIJE KOD INVAZIVNE SLATKOVODNE VRSTE, BABUŠKE *CARASSIUS GIBELIO* (BLOCH, 1782) U TURSKOJ OTKRIVENO SEKVENCIONIRANJEM GENA MITOHONDRIJALNE CITOHRM OKSIDAZE I

### *Apstrakt*

Invazivne vrste riba veoma lako postaju dominantne vrste u slakim vodama i mogu da negativno utiču na stanište (Paulovits et al., 1998). Jedna od najpoznatijih ne nativnih slatkovodnih vrsta u Turskoj je babuška *Carassius gibelio* (Bloch, 1782). Molekularne analize nam daju značajne informacije za bolje razumevanje činjenica vezanih za rastuću bioinva-ziju. Ove molekularne analize izvršene su poređenjem genetskih varijacija vrsta (Doğaç et al., 2015). U ovom istraživanju, naš cilj je da ispitamo genetske varijacije babuške u Turskoj, pomoću mitohondrijalnog gena citohrom oksidaze I (COI).

Uzorci riba izlovljeni su elektroribolovom iz svih krajeva Turske. Tkivo mišića korišće-no je za ekstrakciju DNK. COI amplifikacije izvršene su sa dva para prajmera (Ward et al., 2005). PCR reakcije postavljane su u zapremini od 25 µl, gde je svaka sadržala 2.5 µl 10X Taq Buffer sa KCl (100 mM Tris-HCl, 500 mM KCl, pH 8.8), 2µl MgCl<sub>2</sub> (25mM), 0.5 µl dNTPs (10 mM), 0.5 µl svakog prajmera (10 pM/µl), 2 U of Taq polimeraze (5U/µl) i 2 µl DNK (50 ng/µl). Amplifikacije su izvršene u 'termosajkleru' sledećim uslovima (Keskin and Atar, 2012): preliminarna denaturacija na 95°C u trajanju od 2 minuta, zatim 35 ciklusa koji se sastoje od denaturacije na 95°C u trajanju od 30 sekundi, prvo žarenje i hlađenje na 55°C u trajanju od 30 sekundi, prva ekstenzija na 72°C u trajanju od jednog minuta. Ceo proces završen je krajnjom ekstenzijom na 72°C u trajanju od 10 minuta. Analiza podataka izvršena je sa MEGA 5.0 (Tamura et al., 2011), DNKSP 5.0 (Librado & Rozas, 2009) i Network 4.6 (Bandelt et al., 1999).

Tri haplotipa pronađena su među 220 sekvenci i jedan od tih haplotipa je jedinstven za Tursku. Diverzitet haplotipa bio je 0.27. Diverzitet nukleotida procenjen je kao 0.009.

Mreža haplotipa je pokazala da su haplotipi Turske baubuške blisko povezani sa Japanskom populacijom.

Genetska varijacija populacije babuške u Turskoj je bila niska, ali nivoi genetskog strukturiranja sa novim jedinstvenim vrstama su bili visoki. Rezultati ovog istraživanja pokazuju da je babuška izmeštena u Tursku iz svojih nativnih i ne nativnih krajeva.

*Ključne reči: genetske varijacije, invazivne vrste, haplotipovi, babuška*

## INTRODUCTION

Invasive fishes quite become one of the predominant species in freshwaters and may affect the habitat in a negative way (Paulovits et al., 1998). One of the most prominent non-native freshwater fish species in Turkey is gibel carp *Carassius gibelio* (Bloch, 1782). Molecular analyses provide beneficial information for a better comprehension of the facts required for a thriving bioinvasion by comparing genetic variation of a species (Doğaç et al., 2015). In this study, we aim to investigate genetic variation of gibel carp from Turkey, using mitochondrial cytochrome oxidase I (COI) gene.

## MATERIAL AND METHODS

Fish samples were collected by electrofishing from all parts of Turkey. Muscle tissues used in DNA extraction. Amplifications of COI were carried out using two primer pairs (Ward et al., 2005). PCR reactions were set up in 25 µl volumes, each containing: 2.5 µl of 10X Taq Buffer with KCl (100 mM Tris-HCl, 500 mM KCl, pH 8.8), 2µl of MgCl<sub>2</sub> (25mM), 0.5 µl of dNTPs (10 mM), 0.5 µl of each primer (10 pM/µl), 2 U of Taq polymerase (5U/µl) and 2 µl of DNA (50 ng/µl). Amplifications were conducted in thermal cycler with the following cycling conditions (Keskin and Atar, 2012): preliminary denaturation at 95°C for 2 minutes followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, primer extension at 72°C for 1 minute and concluded by a final extension step at 72°C for 10 minutes. Data analyses were conducted using MEGA 5.0 (Tamura et al., 2011), DnaSP 5.0 (Librado & Rozas, 2009) and Network 4.6 (Bandelt et al., 1999).

## RESULTS

Three haplotypes were detected among 220 sequences and one of these haplotypes is unique for Turkey. Haplotype diversity was detected as 0.27. Nucleotide diversity was estimated as 0.009. Haplotype network showed that haplotypes of Turkish gibel carp populations seemed to be closely related with Japanese populations.

## DISCUSSION

Genetic variation was found to be low for gibel carp populations in Turkey but also showed high level of genetic structuring with new unique haplotype found. Findings of this study specified that gibel carp was translocated to Turkey from both native and non-native area of the species.

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