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GENETIC VARIABILITY IN CULTURED AND WILD POPULATIONS OF *Clarias gariepinus* (Osteichthys: Clariidae) USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKER

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ABSTRACT

Three wild populations of *Clarias gariepinus* from Esaodo (River Osun), Owena (River Owena), and Agbabu (River Oluwa), and three farmed populations, viz: Akure, Ilesa and Ado-Ekiti, in Southwest Nigeria, were analysed for their genetic differences using Random amplified polymorphic DNA (RAPD) analysis. Live specimens comprising 40 individuals *(*680 ± 3.28 g) from each location were collected and kept in six concrete tanks (2x1x1m).

Altogether 435 reproducible bands were obtained from six populations for the nine RAPD primers used. The Analysis of Molecular Variance (AMOVA) indicated that the sampled populations are significantly different from each other, and that 99% of the total variation resided within the population. The percentage of genetic identity (GI) of RAPD-PCR profile among six populations ranged from 74.6% to 83.5%, while Genetic distance of the six populations based on RAPD-PCR profile ranged from 0.180 to 0.293. Estimates of genetic variation in wild and cultured populations of *C. gariepinus* were made, and total and mean number of segregating fragments were 71 (89.9%), 34.5 and 59 (74.7%), 35.4, respectively. Total gene diversity within wild and cultured populations (Ht) was 0.3419 and 0.3010, respectively.

The study established that there is genetic variability in both wild and cultured *C. gariepinus.* RAPD showed that samples within the wild and cultured populations under study were closer to each other than between the two habitats. With reference to total gene diversity values and total number of segregating fragments, the wild population was considerably more diverse than the cultured population.

INTRODUCTION

The degree of genetic variation in a population clearly specifies what kind of changes it might have experienced in the past, what the current situation is and what the probability of sustenance is in future (Rashid et al., 2012). Lynch et al. (1995) and Loew (2000) reported that low levels of genetic diversity have a negative correlation with the potential for adaptation to changing environmental conditions, and that the survival of endangered species may be threatened.

Inbreeding is a common scenario in fish hatcheries (Simonsen et al., 2005) and the offsprings produced which are genetically inferior are used to stock ponds, and in most cases unintentionally escape into river bodies, causing feral gene introgression into pure wild stocks. Van der Walt et al. (1993) reviewed genetic variability in *C. gariepinus* and found strong evidence of inbreeding, founder effects and genetic drift in most captive populations.

Thus *C. gariepinus* may be prone to loss of genetic diversity and variability due to the extinction of genetically distinct wild populations as a result of the escape of hatchery-reared fish or the ranching of fry, as reported by Ponniah (1997) in carps.

Several chemical and biochemical techniques such as Iso-

zyme electrophoresis (Smith et al., 1997; Cagigas et al., 1999), restriction fragment length polymorphism (RFLP) (Hallerman and Beckmann, 1988) and microsatellites (Huang et al., 2000) have been used in analyzing genetic similarity and diversity in genetics and breeding research involving fish and other vertebrates. Random amplified polymorphic DNA (RAPD) has been used to evaluate genetic diversity and similarity in several organisms (Cagigas et al., 1999; Bartish et al., 2000; Huang et al., 2000; Lumaret et al., 2000; Mohd-Azmi et al., 2000; Hwang et al., 2001). Application of RAPD in the study of genetic similarity and diversity was reported in fish (Coughlan et al., 1998; Cagigas et al., 1999). Also, many genetic techniques have been applied in fish species other than *C. gariepinus.* The greatest advantages for RAPD technique are that it can potentially sample a large number of loci and that no prior DNA sequence information is needed to perform the assay (Christopher et al., 2004).

Clarias gariepinus is the most widely cultured catfish in Africa and the third most cultured catfish species in the world (Garibaldi, 1996). It has an almost Panafrican distribution and also naturally occurs in Asia Minor (Skelton and Teugels, 1992).

Increase in hatchery-reared catfish production has necessitated the need to understand the genetic composition of natural catfish populations. This will assists in evaluating the exact latent genetic variation present in many cultured catfish varieties. Despite the commercial importance of the species, there is paucity of information on the population dynamics of clariid fishes. Also, the origin and relatedness of this species are not well elucidated. Moreover, in Nigeria, genetic data on clariid catfish stocks are scarce.

The objective of this study therefore was to characterize the genetic variation and relatedness in wild and cultured *C. gariepinus* populations from Southwest Nigeria using RAPD markers.

MATERIALS AND METHODS

Fish sampling

Geographically isolated populations of *C. gariepinus* were collected from three different rivers, viz: the Oluwa River, River Osun and River Owena in South West Nigeria. The cultured samples were obtained from the Teaching and Research Farm of Federal University of Technology (Akure), Leventis Agricultural Training School, Ilesa (Osun State), Ekiti State Ministry of Agriculture, Ado-Ekiti (Ekiti state) (Figure 1). 240 samples of cultured and wild *C. gariepinus* with average weight of 675 g were obtained from six different locations for RAPD analysis. Blood samples were obtained from the caudal vein of the fish using heparinized syringe and preserved in 95% ethanol.

DNA isolation and extraction

Genomic DNA was extracted from the blood samples following a proteniase K-phenol chloroform-based protocol, as described by Ruzzante et al. (1996) at the Biotechnology Centre, Federal University of Agriculture, Abeokuta, Nigeria. The purity and concentration of genomic DNA were determined by calculating the ratio of the optical density measured at 260/280 nm with a spectrophotometer. DNA samples from individuals of each population were diluted to approximately 25 ng mL-1 with distilled water before being used for PCR amplification. The extracted DNA was stored at -20°C for 3 days.

PCR amplification and electrophoresis

A series of optimization experiments were conducted using the protocol described by Williams et al. (1990) to determine which conditions produced the strongest and most reproducible patterns.

Twenty commercially available decamer primers (OPAE4-9, OPAD3-9 and OPAF6-12) from Operon technologies (Alameda, CA, USA) were used for this study. The amplification reactions were performed in volumes of 25uL, containing 50µg of genomic RAPD, 2mM MgCl2, 100uM of dATP, dCTP dGTP and dTTP each, 0.2um of the primer and 0.5 units of Taq DNA polymerase. The total volume of the PCR products were evaluated in 2% agarose gels and visualized by ethidium bromide staining. After electrophoresis, DNA bands profiling were observed under UV light, and the images were documented. The pictures from the gel were used for the analysis of the amplified products.

Data analysis

Amplified fragment was scored as binary data, i.e. presence as 1 and absence as 0. Only data generated from reproducible bands were used for statistical analysis. The number of polymorphic loci, percentage of polymorphic loci (%P), observed number of alleles (ne) and Nei's gene diversity (H) were estimated using the AFLP-SURV v 1.0 software with Bayesian approach with non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999), which gives an unbiased estimate of allele frequencies from RAPD data (Zhivotovsky, 1999). Genetic distance and identity were estimated using GenAlEx 6.41 software. The Tools for Population Genetic Analysis (TFPGA) software v. 1.3 (Miller, 1997) was applied for the estimation of population differentiation by the Reynolds' coancestry coefficient (Fst) (Reynolds et al., 1983). It was also used to construct an unweighted pair-group method with average (UPGMA) dendrograms (Sneath and Sokal, 1973) based on the estimated genetic distances.

RESULTS AND DISCUSSION

Nine primers (OPAD-09, OPAE-04, OPAE-05, OPAE-09, OPAF-07, OPAF-08, OPAF-09, OPAF-11 and OPAF-12), out of the 20 primers tested, generated reproducible bands. The other 11 primers did not amplify or produce highly inconsistent amplification products from the same individual, and hence the primers were excluded from further analysis. A

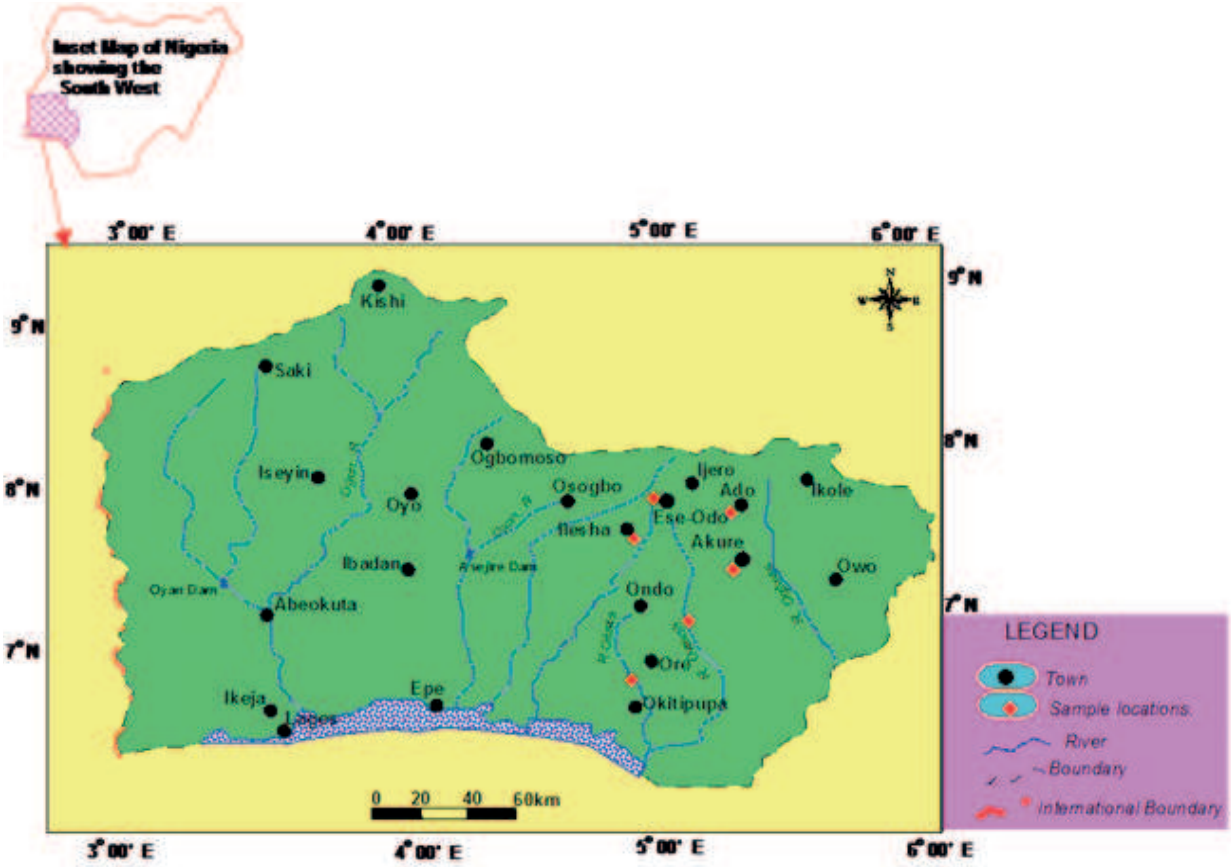

Fig 1. Map of South West Nigeria showing wild (Esaodo, Agbabu and Owena) and cultured (Ilesa, Ado-Ekiti and Akure) sample locations

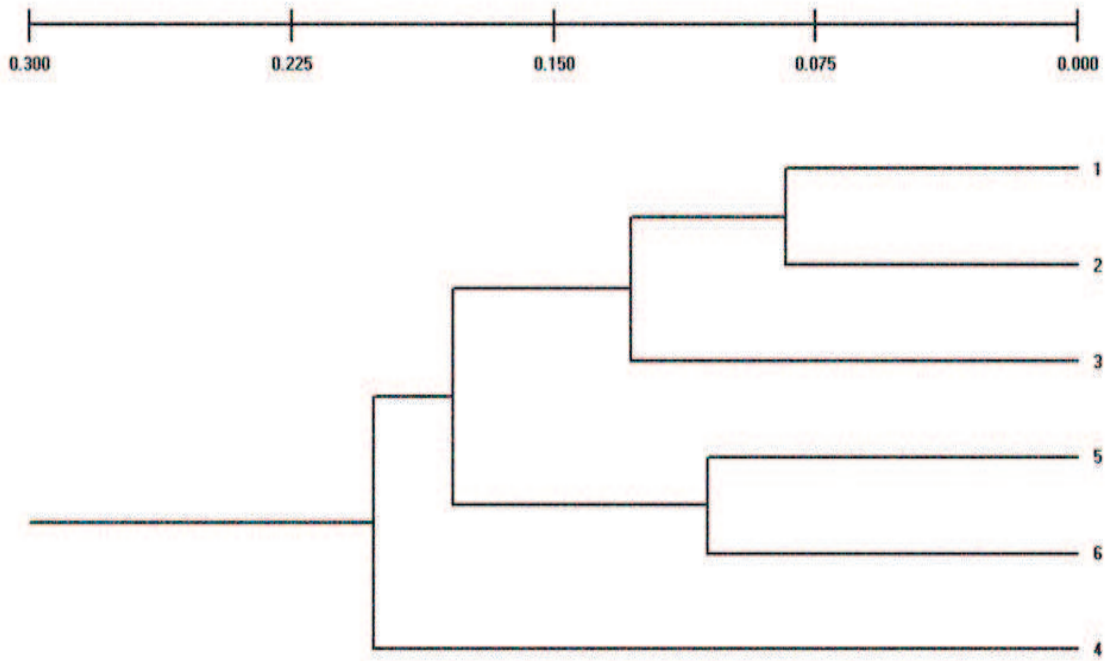

Fig 2. Unweighted pair-group method with average (UPGMA) dendrograms based on Nei's D value (Nei, 1972), original measures of genetic distance, summarizing the data on differentiation between *Clarias gariepinus* populations according to RAPD analysis (1=Akure, 2=Ilesa, 6=Ado-Ekiti (cultured); 3=Owena, 4=Agbabu, 5=Esaodo (wild))

total of 425 reproducible bands were obtained in six populations for nine primers (Table 1). The number of amplified bands varied from 6 to12, with a size range between 150 and 5500 bp.

Table 1. Size and molecular weight of fragments amplified by different random amplified polymorphic DNA primers

S/N	Primer Name	Sequence (5' to 3')	Size range	%GC content	Mol Weight (g/M)
1	OPAD-09	TCGCTTCTCC	200bp - 1500bp	60	2930
2	OPAE-04	CCAGCACTTC	250bp - 2500bp	60	2948
3	OPAE-05	CCTGTCAGTG	150bp - 2000bp	60	3017
4	OPAE-09	TGCCACGAGG	200bp - 2000bp	70	3050
5	OPAF-07	GGAAAGCGTC	250bp - 3000bp	60	3074
6	OPAF-08	CTCTGCCTGA	150bp - 2500bp	60	2978
7	OPAF-09	CCCCTCAGAA	200bp - 5500bp	60	2957
8	OPAF-11	ACTGGGCCTC	200bp - 2500bp	70	3002
9	OPAF-12	GACGCAGCTT	200bp - 3500bp	60	3026

The AMOVA indicated that the sampled populations are significantly different from each other ($p<0.05$), and that 99% of the total variation resided within the population.

The phylogenetic similarity between and within the six populations of *C. gariepinus* is depicted by UPGMA dendrogram based on Nei's genetic distance (Nei, 1972) (Figure 2). The dendrogram separated the six *C. gariepinus* populations into two distinct clades, Agbabu population being alone in one clade, while Owena, Esaodo and hatchery populations belonged to the other clade. The second clade was further separated into two subgroups, Ado-Ekiti and Esaodo populations in one sub-group, and Akure, Ilesa and Owena populations in the other subgroup.

The genetic distance and identity of *C. gariepinus* from six populations (Table 2) based on RAPD-PCR profile ranged from 0.180 in Akure/Ilesa to 0.293 in Esaodo/Owena/Ilesa. The genetic identity of *C. gariepinus* from six populations also ranged between 0.746 in Esaodo/Ilesa/Owena and Akure/Ilesa, respectively.

The total gene diversity (H_t), mean gene diversity within populations (H_w) and average gene diversity between (H_b) populations exceeding that observed within populations are presented in Table 3.

The proportion of segregating fragment, total and mean gene diversity in wild population of *C. gariepinus* were 89.9%, 0.34 and 0.31, respectively. The higher values were recorded in cultured *C. gariepinus* for the mean number of fragments (35.4), genetic differentiation among populations (0.06) and proportion of gene diversity (0.2) (Table 3).

Table 2. Genetic Distance (below) and Genetic Identity (above diagonal) between the populations of *Clarias gariepinus* based on RAPD-PCR

Akure	Ilesa	Owena	Agbabu	Esaodo	Ado-Ekiti	
-	0.835	0.802	0.804	0.804	0.824	Akure
0.180	-	0.814	0.786	0.746	0.824	Ilesa
0.220	0.206	-	0.834	0.746	0.758	Owena
0.204	0.241	0.181	-	0.761	0.822	Agbabu
0.218	0.293	0.293	0.274	-	0.766	Esaodo
0.193	0.194	0.278	0.196	0.266	-	Ado-Ekiti

RAPD fragments observed in the 240 individuals showed a reasonable degree of genetic diversity within and between the populations. Nine random primers for DNA fingerprinting of *C. gariepinus* generated a total of 425 bands from the 240 individuals in the six populations. 89.9% of wild and 74.7% of cultured *C. gariepinus* were polymorphic. The percentage of polymorphic loci was higher than that observed in the same specie by Saad et al. (2009) in Egypt, which was 69.5%. This might be due to the level of cultivation or high level of mixing between the wild and cultured *C. gariepinus*. The values for wild and cultured *C. gariepinus* fall between those reported by Garg et al. (2010) in *C. batrachus* (86.66%) in India, and in *H. fossilis* (83.87%) (Sultana et al., 2010) in India. However, the value was higher than those in two populations of *H. fossilis* (18.75%) reported by Garg et al. (2009), and three populations of *C. batrachus* (25-35.5%) reported by Khedkar et al. (2010) in India. Hassanien (2008) reported polymorphic of 44% to 64% in five populations of *D. labrax* (L.). A higher value recorded in this study might be due to species differences.

A higher percentage of polymorphic loci obtained in the wild population of *C. gariepinus* (89.9%) indicated a relatively higher level of genetic variation. However, a lower percentage of polymorphic loci (74.7%) in the cultured population could be an indication of inbreeding in the hatchery population compared with the respective natural populations. The lowest percentage of polymorphic loci (64.52%) was also reported in the hatchery population of *H. fossilis* (Sultana et al., 2010), *C. catla* (Rahman et al., 2009) and *L. rohita* (Islam and Alam, 2004) using RAPD marker analysis.

High quality bands with good reproducibility generated

Table 3. Estimates of genetic variation in wild and cultured populations of *Clarias gariepinus*

Populations	Total no. of segregating fragments	Mean no. of fragments per individual	H_t	H_w	H_b	F_{st}
Wild	71 (89.9%)	34.50	0.34	0.31	0.04	0.11
cultured	59(74.7%)	35.40	0.30	0.24	0.06	0.20

H_t: total gene diversity among populations; H_w: mean Nei's gene diversity within populations, H_b: genetic differentiation among populations and F_{st}: the proportion of the total gene diversity that occurs among, as opposed to within populations

in this study fall within the size range of 250-2700bp. The sizes of reproducible RAPD markers reported by Liu et al. *(*1999) ranged from 200 to 1500bp in *I. punctatus.* Ambak et al. (2006) reported 200 to 1500 in *Channa striata*. Similar observation was made by Garg et al. (2010) who reported 172 to 1677bp in *C. batrachus*. Khedkar et al. (2010) found good quality bands in the range of 100 to 1200bp in *Clarias batrachus*. The higher value in band range in this study might be attributed to species differences and source of samples.

Genetic identity measures in the pairwise of populations show the proportion of individuals of the six populations that are genetically identical. A higher genetic identity (0.835) between Ilesa and Akure populations of *C. gariepinus* indicates that they are genetically more similar than the other populations. The reason for this higher value may be that the parent stock might probably have a common or closer source, and that the two populations are cultured. This value is lower than the genetic identity value (0.939) obtained between the two wild populations of *H. fossilis* (Sultana et al., 2010). The lower genetic identity values between Agbabu and Esaodo (0.761) indicate that the two populations are genetically different. The lower identity value between Agbabu and Esaodo might be due to the possibility of intermixing in the natural environment, thus reducing genetic identity. The genetic identity values agree with the expected values for the individuals of the same population. This was supported by the findings of Thorpe and Sole-Cava (1994) who found that 98% of populations of the same species have a genetic similarity above 0.85.

There were variations in genetic distance in the six populations. The lowest genetic distance (0.180) was found between Akure and Ilesa populations, and the highest (0.396) was found among Ilesa, Esaodo and Owena populations.

In the present study, there was no significant correlation between genetic identity and geographical distance.

Genetic diversity within a population is highly important for adaptation to changing environments, as consequences, for long term survival of species. In this study, the populations of *C. gariepinus* revealed a slightly higher within-population variation in wild populations compared to the cultured populations. This could be related to a huge floodplain along these rivers and their tributaries. In the rainy season, the floodplain provides a breeding ground and in the dry season it serves as shelter, thus enabling high effective population sizes. This may enhance interbreeding between populations that occupy this region, thus increase genetic variation within population.

Genetic variation between populations was compared from the dendrogram. Agbabu, an isolated population, was far from the other populations studied and this resulted in large genetic distances between Agbabu and the other five populations. The lower genetic distance among Akure, Ilesa and Esaodo population pairs might suggest that the farmers in Akure and Ilesa obtained their brood fish from Esaodo. A lower genetic distance noticed in Esaodo and Ado-Ekiti populations could be due to the escape of some samples from Ado-Ekiti through flooding which carried them to Esaodo. This will aid intermixing of the fish from the two populations, resulting in high level of gene flow and inter-population similarities between both populations. The wild population was more diverse than the cultured population based on the total gene diversity values and total number of segregating fragments.

It was demonstrated in this study that RAPD markers can differentiate between and determine genetic relatedness within populations of *C. gariepinus.* This is a relevant baseline information regarding the genetic variation and population structure before embarking on any breeding programme.

The genetic variation in the hatchery populations was slightly lower than that of the wild populations. As the culture of clariid catfish is gaining popularity and expanding rapidly, and additional hatcheries are being engaged in fish breeding, serious attention has to be paid to avoid inbreeding and genetic drift that may ruin the genetic variability and thus biological potential of *C. gariepinus* in Southwest Nigeria.

Sažetak

ISTRAŽIVANJE GENETSKE VARIJABILNOSTI UZGOJENIH I DIVLJIH POPULACIJA SOMA, *Clarias gariepinus* (Osteichthys: Clariidae), POMOĆU MARKERA SLUČAJNO UMNOŽENE POLIMORFNE DNA (RAPD)

Analiza slučajno umnožene polimorfne DNA (RAPD) korištena je pri istraživanju genetske sličnosti i različitosti između tri uzgajane populacije i tri populacije iz otvorenih voda vrste *Clarias gariepinus* iz jugozapadne Nigerije.

Genetska varijabilnost je analizirana kod tri populacije iz otvorenih voda Esaodoa (rijeka Osun), Owene (rijeka Owena) i Agbabua (rijeka Oluwa) i kod triju uzgajanih populacija: Akurea, Ilese i Ado-Ekitija. Sa svake lokacije prikupljeno je četrdeset živih jedinki *(*680 ± 3,28 g).

Dobiveno je ukupno 435 produciranih crta iz šest populacija korištenjem devet RAPD početnica. Analiza molekularne varijance AMOVA ukazuje da se istraživane populacije međusobno značajno razlikuju i da se 99% ukupne varijabilnosti tiče različitosti unutar populacija. Postotak genetske istovjetnosti (GI) s RAPD-PCR analizom između šest proučavanih populacija kretao se od 74,6% do 83,5%. Također, genetska udaljenost vrste *C. gariepinus* iz šest populacija, temeljena na *RAPD-PCR* analizi, kretala se od 0,180 do 0,293. Napravljene su procjene genetskih varijacija kod populacija iz otvorenih voda i uzgajanih populacija vrste *C. gariepinus*, a ukupna i srednja vrijednost izdvojenih fragmenata bila je 71 (89,9%), 34,5 i 59 (74,7%), 35.4.

Ukupna genska različitost između populacija iz otvorenih voda i uzgajanih populacija (Ht) bila je 0,3419 i 0,3010.

Zaključeno je da postoji genetska varijabilnost i kod populacija iz otvorenih voda i kod uzgajanih populacija vrste *C. gariepinus.* Također, rezultati RAPD analize pokazali su međusobnu veću bliskost proučavanih jedinka populacija iz otvorenih voda i uzgajanih populacija nego onih između dvaju staništa. S obzirom na ukupne vrijednosti genske raznolikosti i ukupnog broja izdvojenih fragmenata, populacije iz otvorenih voda su znatno različitije nego uzgajane populacije.

Ključne riječi: *Clarias gariepinus*, RAPD, genska različitost, genetska varijacija

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