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GENETIC ANALYSIS OF THREE GEOGRAPHICALLY SECLUDED POPULATIONS OF NILE TILAPIA *Oreochromis niloticus* (CICHLIDAE)

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ABSTRACT

Nile tilapia *Oreochromis niloticus* is one of the most important fishery resources and a valuable fish species for aquaculture programmes. It is found in almost all waters and is widely dispersed. Several natural populations of this species have been impacted by genetic pollution despite their significant economic relevance. Understanding population structure is a crucial first step in protecting this species in its native habitats as well as in choosing which wild stocks to use in hatchery initiatives. To demonstrate the genetic-population structure of this species, genetic differences among three geographically secluded populations of *O. niloticus* were investigated utilizing mitochondrial DNA cytochrome b gene sequences. The results were used to estimate the levels of genetic variability within and among the populations. The 56 cyt b (821 bp) sequences analysis revealed 21 haplotypes, with a nucleotide diversity of 0.0510 and a haplotype diversity of 0.881. In each of the populations, seven (7) singleton variable sites and 19 informative-parsimony sites, genetic diversity could be identified and few population haplotypes were found, indicating a minor genetic distinction between them. For the purpose of conservation and/or Nile tilapia breeding programmes, this information would assist in choosing the fish populations that maintain greater genetic variation in *O. niloticus*.

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INTRODUCTION

In the world's fisheries and aquaculture production, Nile tilapia *O. niloticus* appears to be the fish with the greatest economic significance (Bostock et al., 2010; Josupeit, 2010). For aquaculture purposes, the species has been introduced practically to every tropical and subtropical environment (Mjoun and Rosentrater, 2010), The fish is the most widely accepted and prevalent genus in Nigeria among cichlids (Mert and Cicek, 2010). This is due to its good aqua-farming characteristics, such as its capacity to endure unfavourable water conditions and its ability to accept diverse feed materials (Yakubu and Okunsebor, 2011). Nile tilapia is rich in high-protein, low-fat, sources of essential nutrients such as potassium, phosphorus, and vitamin B-12, all of which are necessary for body growth and development (Shahriar et al., 2011).

Continuous contamination of water sources, escape from the hatchery and indiscriminate spawning of *O. niloticus* have the potential to cause genetic degradation in this species over time. Hybridization between exotic and native *O. niloticus* species is thus a key concern for wild species conservation (D'Amato et al., 2007). This could lead to local species losing their genetic purity, resulting in the extinction of natural species. Additionally, this might cause the loss of pure species as a result of transgressive segregation and the emergence of hybrid swarms (Crispo et al., 2011). Furthermore, hybridization impairs the parental population's fitness and reproductive success (Muhlfeld et al., 2009), resulting in low fecundity and, as a result, decreased fish yields (Amarasinghe and De-Silva, 1996). Increased anthropogenic activity in ecological processes can also play a significant role in genetic exchange across fish populations, resulting in genetic polymorphism in the species' native range (Crispo et al., 2011).

Comparative genomic studies which assess population variance in terms of allelic polymorphisms have increasingly become an important aspect of the agricultural approach required in selecting brooders and identifying threatened species for conservation efforts (Mert and Cicek, 2010). Diverse DNA signatures are characterization tools in studies of aquatic species to assess hereditary inconstancy within or among populations; these include allozymes (Agnèse et al., 1997; Moralee et al., 2000; Rognon and Guyomard, 2003), and restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPDs) (Ali et al., 2004; Popoola et al., 2014). However, there is a dearth of information on mtDNA markers for *O. niloticus* in the study areas. Mitochondrial DNA (mtDNA) markers are quite diverse and show a trend towards maternal ancestry. They have been employed to assess phylogenetic connections and genetic differences between various fish populations and species (Faulks et al., 2008; Ferguson and Danzmann, 1998). For research on the population of different types of fish, researchers frequently use mitochondrial DNA markers like cyt b genes (Habib et al., 2012). A past study using cyt b to quantify genetic diversity in *Labeo rohita* populations by Luhariya et al. (2011) showed that this marker can be used to assess genetic disparity in both intra and inter-populations. Three populations of *O. niloticus* were studied, and the DNA strands that make up the cyt b genes were compared to estimate the genetic diversity among the studied populations. The findings offer an important insight into the current genetic state of Nile tilapia *O. niloticus*. This information will assist in the formulation of an appropriate management approach and stock enhancement programmes.

MATERIALS AND METHODS

Nile tilapia *O. niloticus* (n = 56) were collected from three locations (Asejire, Ureje, and Ogbese River in Oyo, Ekiti, and Ondo in Nigeria) between May and October 2019. The fish were transported in boxes containing ice appropriate for DNA extraction.

Adopting the phenol/chloroform technique described by Ullrich et al. (1977), genomic DNA from 56 fish was extracted from caudal fin clips. An agarose gel electrophoresis (1.0% w/v) was used to ascertain the quality of DNA. A NanoDrop Spectrophotometer (NanoDrop™ ND-1000, Thermo Scientific, Inc.) was used to determine the concentration and purity of the DNA.

The primers cyt b F 5' TGACTTGAAAAACCACCGTTGTTA 3' and cyt b R: 5' CTTCGGTTTACAAGACCG 3' were employed to amplify a 1227 bp (1.2 kbp) portion from the mitochondrial regulatory region. Molecular grade water of 21.25 µl, 1.5 µl of each primer (10 mM), 1.5 µl of dNTPs (10 mM), 0.375 µl of Taq polymerase, 1 µl of template DNA were added to 3.0 µl of 10X PCR buffer and placed into a 30 µl tube to run the Polymerase Chain Reactions (PCRs) in 30 cycles at an annealing temperature of 53 °C, 30 seconds, followed by denaturation temperature of 94°C for 30 seconds, initial extension temperature (72 °C for 1 min), and a final extension at 72°C for 10 mins, and subsequently to the initial 5 minutes denaturation at 94 °C. The Inqaba DNA Purification Kit (Inqaba Biotech, South Africa) was used to complete the PCR amplification, which was subsequently stained using ethidium bromide stain on 1.0% agarose gels (Johannesburg, SA). The purified DNA was sequenced for bidirectional sequencing using PCR primers by Inqaba Biotech in Johannesburg, South Africa.

Data analysis

Molecular Evolutionary Genetics Analysis (MEGA 11.0) was used to compare and align the DNA sequences from each specimen collected in this study with Clustal W. Sequence comparisons, pairwise genetic distance

estimation, and neighbour joining (NJ), using the Kimura 2-parameter (K2P) distance model (Tamura et al., 2011). Arlequin's analysis of molecular variance (AMOVA) was used to determine the population structure of *O. niloticus* (Excoffier et al., 1992). The DNAsp v. 6.5 was used to determine how genetic variations affect traits, as in the diversity of nucleotides (p) and haplotypes (h) (Rozas et al., 2003). The PopART 1.7 software was used in creating a haplotype network (Bandelt et al., 1999), while calculations of sequence composition, indexes of molecular diversity, and genetic differentiation were made with Arlequin 3.11 (Excoffier and Lischer, 2006). The support for bootstraps was computed using 1000 replications, and the mean genetic distances across *O. niloticus* populations were estimated using the same technique.

RESULTS

Cytochrome b region's sequence analysis identified 26 polymorphic sites, 21 haplotypes, and 19 parsimony informative sites. In different populations, different haplotypes were discovered at different frequencies. The Asejire *O. niloticus* population had the most haplotype diversity (8 haplotypes), whereas the Ureje and Ogbese *O. niloticus* populations had just 7 haplotypes (Table 1). Three sampling sites had haplotype diversity values between 0.72381 and 0.90476, while Pi was between 0.00179 and 0.00537 (Table 1).

The 45 mtDNA cyt b gene haplotypes obtained from the analysis were deposited in the genebank and given the accession numbers (OM811751 - OM811795). The genetic distance for the three populations under study revealed that the Asejire and Ureje *O. niloticus* populations had the highest genetic distance, whereas the Ogbese and Ureje populations had the lowest genetic diversity (Table 2).

According to the AMOVA (Table 3), there was 93% variation between populations and just 7% variation within populations. The pairwise FST values between the three populations demonstrated a high level of variation (0.932).

Table 2. Nei genetic distance for *Oreochromis niloticus* populations

	Asejire	Ureje	Ogbese
Asejire	0.00000		
Ureje	0.15595	0.00000	
Ogbese	0.15560	0.00683	0.0000

The relationship and genetic relatedness of *O. niloticus* from the three populations were shown by phylogenetic investigation. Figure 1 shows the neighbour-joining evolutionary history of three populations of *Oreochromis niloticus*. The neighbour-joining network approaches for trees revealed three major clades with the Asejire population having two clades, while the Ureje and Ogbese populations shared one clade. The procedures produced trees that characterised species from the same genera but placed them into sister clades based on populations (Fig. 1).

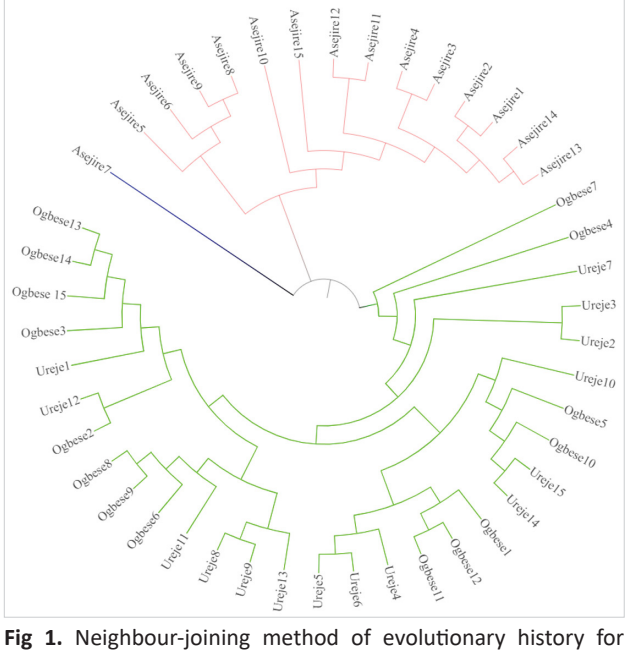

Fig 1. Neighbour-joining method of evolutionary history for three populations of *Oreochromis nilotucus*

Table 1. Genetic analysis, nucleotide frequencies, and the average cyt b sequence in Nile tilapia *O. niloticus* from three populations

Population	Number of sequences	Number of polymorphic sites	Total num-ber of mu-tations	Nucleotide diversity (Pi)	Number of haplotypes (h)	Haplotype diversity (Hd)
Asejire	15	12	12	0.00537	8	0.90476
Ureje	15	7	7	0.00221	7	0.78095
Ogbese	15	5	5	0.00179	7	0.72381
Total	45	26	26	0.00510	21	0.88100

Singleton variable sites 7; Parsimony informative sites 19

Table 3. Genetic variations among and within three Nile tilapia populations (analysis of molecular variance)

Variations	Degree of freedom	Sum of square	Mean Square	Estimated variance.	%
Among populations	2	1425.01	712.50	38.25	93%
Within populations	43	148.94	3.46	2.81	7%
Total	45	1573.95		41.06	100%
FST	0.932	0.010			
Tajima's D statistic	0.86				

The network (Fig. 2) created from sequences from the cyt b gene revealed that three populations of *O. niloticus* formed various clusters and shared similarities between specific haplotypes, which were separated by 1 - 8 mutational steps.

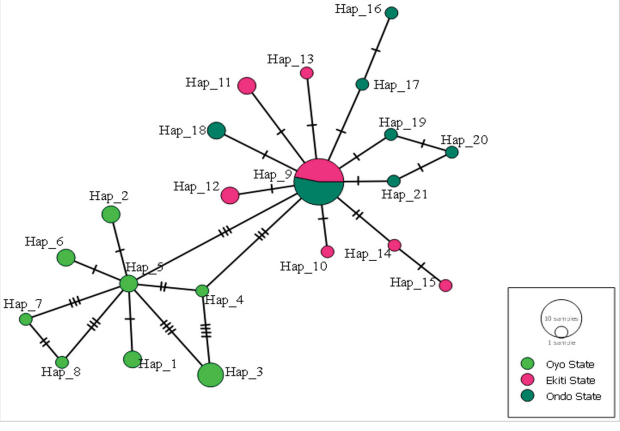

Fig 2. A haplotype structure of the cytochrome b gene region in *O. niloticus*. The parallel bars represent the number of mutations.

DISCUSSION

Due to a lack of rules for character selection or coding in traditional taxonomy, it can occasionally be challenging to distinguish between species. Morphomeristic datasets may be somewhat arbitrary. In these cases, the genetic analysis might be used with other techniques to establish taxonomic genuineness. The cyt b gene has been shown to make species identification easier and to help resolve taxonomic confusion (Behera et al., 2015). Following the current research, *O. niloticus* populations from three different bodies of water in Nigeria displayed significant genetic diversity. It was found that the amplified cyt b regions in the *O. niloticus* population under study are polymorphic. The characteristics of the fish cyt b sequence are consistent with the fact that the majority of the mutations occurred in the third position (Perdices et al., 2004). The 19 parsimony informative sites, 821 constant sites, and 26 variable sites observed in this study were similar to the findings in the Chelidoperca genus from the Indian Ocean reported by Sati et al. (2015).

Populations with large populations and diverse environments can sustain high haplotype diversity (Hd) (Avise et al., 1997; Nei and Miller, 1990). The average quantity of differences between each pair of haplotypes within a population is known as nucleotide diversity (π). Consequently, π values are accurate indicators of genetic diversity in populations (Nei and Li, 1979). Low nucleotide diversity and high Hd in fish are likely to have resulted from population growth following an interval of low effective population size. *Oreochromis niloticus* groups had high Hd values (0.99286) and reduced nucleotide diversity (π) (0.06971), demonstrating that the populations under study had recently diverged (Grant and Bowen, 1998). It is reasonable to conclude that the complex and breeding nature of *O. niloticus* population distributions is responsible for the high level of average Hd reported in South West Nigeria. Numerous factors, such as habitat fragmentation and occupation, bottleneck effects, founder effects, and low nucleotide diversity also imply poor population diversity (Fennando et al., 2000), thus having an impact on genetic diversity. The three populations might have been subjected to more anthropogenic activities, including long-term overfishing since all the fish populations were collected close to residential neighbourhoods. These circumstances would have negatively affected the population structure of *O. niloticus* by diminishing population levels and genetic diversity. The extents of divergence in three populations were compared using the fixation index (Fst). The Fst value (0.932) was found to be significant ($P < 0.05$), along with the AMOVA result which was thought to be crucial for genetic structure in studied populations demonstrating the divergence of haplotypes in *O. niloticus*. Because of habitat loss and impoundments, many species are regionally dispersed. The ratios of the various elements of variability within and between populations gradually vary as dispersal and gene flow decrease. When isolated colonies are subjected to genetic drift, their heterozygosity eventually declines

while their differences widen (Vrijenhoek, 1998). The Fst values observed in this study demonstrate that nucleotide diversity estimates were accurate and that fish from each population considerably differed from one another, demonstrating their genetic distinctiveness. In contrast to movement and anthropogenic activities, geographic isolation, unpredictable environmental conditions and the rapid decline of a colony can cause increased divergence (Lehmann et al., 1998). It is widely recognised that dispersed populations, especially freshwater fish species, are likely to exhibit high genetic diversity (Ward et al., 1994). Such considerable intraspecific variation could have been due to *O. niloticus* populations being most likely to split, and the population under study might have diverged from a common ancestor/population to evolve independently (Grant and Bowen, 1998).

Three populations of *O. niloticus* clustered together in phylogenetic trees built with neighbour-joining (NJ) dendrogram. The evolutionary link between the river bodies was established, with comparable individuals clustered under the same node and dissimilar individuals clustered under different nodes.

The most prevalent haplotype in cyt b was Hap 9, which had a large number of linking nodes in the haplotype network. Because of the strong relationship between haplotype sites in the cyt b gene, it is most likely that the ancestral haplotype, either directly or indirectly through mutation, gave rise to other haplotypes. In the natural populations of *O. niloticus*, the results showed polymorphism as well as the suitability of fragmented cyt b mtDNA sequences for identifying intraspecific genetic variation and separating genetic stocks.

CONCLUSIONS

In recent years, there has been an abrupt decline in the number of wild populations of *Oreochromis nilotics*, regardless of the species' significant economic value as a fishery resource and asset in aquaculture initiatives. Programmes aimed at maintaining and recovering species can have a higher chance of success if suitable natural stocks are carefully chosen by considering genetic variables. The current *O. niloticus* mtDNA data may be beneficial in identifying founder wild stocks of the species that are more variable in terms of DNA. Additionally, given that additional genetic data are crucial in establishing a precise picture of the distribution and dynamics of *O. niloticus* populations in South West Nigeria, it would be instructive to carry out the genetic characterization and monitoring of the species' natural and captive stocks using additional molecular markers in the years to come.

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GENETIČKA ANALIZA TRI ZEMLJOPISNO UDALJENE POPULACIJE NILSKE TILAPIJE *Oreochromis niloticus* (CICHLIDAE)

SAŽETAK

Jedan od značajnih ribolovnih resursa i vrijednih akvakulturnih ribljih vrsta je nilska tilapija, *Oreochromis niloticus*. Nalazi se u gotovo svakoj vodi ovog područja i široko je rasprostranjena. Nekoliko prirodnih populacija ove vrste bilo je pod utjecajem genetskog onečišćenja unatoč njihovoj značajnoj ekonomskoj važnosti. Razumijevanje strukture njezine populacije presudan je prvi korak u zaštiti ove vrste u njezinim izvornim staništima, kao i u odabiru čistih zaliha koje će se koristiti u mrijestilišta. Kako bi se utvrdila genetsko-populacijska struktura ove vrste, genetske razlike između triju geografski izoliranih populacija *O. niloticus* istraživane su korištenjem sekvenci gena citokroma b, mitohondrijske DNK. Rezultati su korišteni za procjenu razine genetske varijabilnosti unutar i među populacija. Analiza sekvenci od 56 cyt b (821 bp) otkrila je 21 haplotip, s 0,0510 nukleotidnom raznolikošću i 0,881 haplotipskom raznolikošću. U svakoj od populacija, sedam (7) mjesta imaju singleton varijable i 19 parsimonijskih informativnih mjesta, genetička raznolikost se mogla identificirati, a pronađeno je samo nekoliko populacijskih haplotipova, što ukazuje na manju genetsku razliku među njima. U svrhu očuvanja i/ili programa uzgoja nilske tilapije, ove informacije bi mogle pomoći pri odabiru ribljih populacija koje će održavati veću genetsku varijaciju kod *O. niloticus*.

Ključne riječi: genetska karakterizacija, mitohondrijska DNA, citokrom b, frekvencije nukleotida

REFERENCES

Agnèse, J. F., Adepo-Gourene, B., Abban, E. K., Fermon, Y. (1997): Genetic differentiation among natural populations of Nile tilapia *O. niloticus*. Heredity, 79, 88-96.

Ali, B. A., Huang, T. H., Qin, D. N., Wang, X. M. (2004): A review of random amplified polymorphic DNA (RAPD) markers in fish research. Reviews in Fish Biology and Fisheries, 14, 4, 443-453.

Amarasinghe, U. S., De-Silva, S. S. (1996): Impact of *Oreochromis mossambicus x O.niloticus* (Pisces, Cichlidae) hybridization on population reproductive potential and long-term influence on a reservoir fishery. Fisheries Management and Ecology, 3, 239-249.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T, Neigel, J. E., Reeb, C. A., Saunders NC (1987) Intraspecific phytogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology, Evolution, and Systematics, 18, 489–522.

Bandelt, H. J., Forster, P., Ro¨hl, A. (1999): Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16, 37–48.

Behera, B. K., Singh, N. S., Paria, P., Sahoo, A. K., Panda, D., Meena, D. K., Das, P., Pakrashi, S., Biswas, D. K., Sharma, A. P. (2015): Population genetic structure of Indian shad, *Tenualosa ilisha* inferred from variation in mitochondrial DNA sequences. Journal of Environmental Biology, 36, 5, 1193–1197.

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., Corner, R. (2010): Aquaculture: Global status and trends. Philosophical Transactions of the Royal Society B, 365, 2897-2912.

Crispo, E., Moore, J. S, Lee-Yaw, J. A., Gray, S. M., Haller, B. C. (2011): Broken barriers: Human-induced changes in gene flow and introgression in animals: An examination of the ways in which humans increase genetic exchange among populations and species and the consequences for biodiversity. Bioessays, 33, 508-518.

D'Amato, M. E., Esterhyse, M. M., Van der Waal, B. C. W., Brink, D., Volckaert, F. A. M. (2007): Hybridization and Phylogeography of the Mozambique Tilapia *Oreochromis mossambicus* in Southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. Conservation Genetics, 8, 475-488.

Excoffier, L., Lischer, H. (2006): An integrated software package for population genetics data analysis. Computational and Molecular Population Genetics Lab (CMPG), Institute of Zoology, University of Berne, Switzerland.

Excoffier, L., Smouse, P. E., Quattro, J. M. (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131, 479–91.

Faulks, L. K., Gilligan, D. M., Beheregaray, L. B. (2008): Phylogeography of a threatened freshwater fish (*Mogurnda adspera*) in eastern Australia: Conservation implications. Marine and Freshwater Research, 59, 89–96.

Fennando, P., Pfrender, M. E., Encalada, S. E., Lande, R. (2000): Mitochondrial DNA variation, phylogeo,graphy and population structure of the Asian elephant. Heredity, 84, 362–72.

Ferguson, M. M., Danzmann, R. G. (1998): Role of genetic markers in fisheries and aquaculture: Useful tools or stamp collecting. Canadian Journal of Fisheries and Aquatic Sciences, 55, 1553–63.

Grant, W. S., Bowen, B. W. (1998): Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. Journal of Heredity, 89, 415–426.

Habib, M., Lakra, W. S., Mohindra, V., Lal, K. K., Punia, P., Singh, R. K., Khan, A. A. (2012): Assessment of ATPase 8 and ATPase 6 mtDNA sequences in genetic diversity studies of *Channa marulius* (Channidae: Perciformes). Proceedings of the national academy of sciences, 82, 4, 497-501.

Josupeit, H. (2010): World supply and demand of tilapia 2010. Paper presented at 3rd International Technical and Trade Conference and Exposition on Tilapia, 27-29.

Lehmann, T., Hawley, W. A., Grebert, H., Collins, F. H. (1998): The effective population size of Anopheles gambiae in Kenya: implications for population structure. Molecular Biology and Evolution, 15, 3, 264-276.

Luhariya, R. K., Lal, K. K., Singh, R. K., Mohindra, V., Punia, P., Chauhan, U. K., Gupta, A., Lakra, W. S. (2012): Genetic divergence in the wild population of *Labeo rohita* (Hamilton, 1822) from nine Indian rivers, analyzed through mtDNA cytochrome b region. Molecular Biology Reports, 39, 4, 3659–3665.

Mert, R., Cicek, E. (2010): Range expansion of introduced tilapia species (*O. niloticus*, L. 1758, Cichlidae) in Turkey. Journal of Animal and Veterinary Advances, 9, 1753-1756.

Mjoun, K., Rosentrater, K. A. (2010). Tilapia: Profile and economic importance. South Dakota: South Dakota Cooperative Extension Service, pp 205

Moralee, R. P., Van der Bank, F. H., Van da Waal, B. C. W. (2000): Biochemical genetic markers between the endemic *Oreochromis mossambicus* and alien species (pisces: Cichlidae). Water SA 26:2c.

Muhlfeld, C. C., Kalinowski, S., McMahon, T. E., Taper, M. L., Painter, S., Leary, R. F., Allendorf, F. W. (2009): Hybridization rapidly reduces the fitness of native trout in the wild. Biology Letters, 5, 328-331.

Nei, M., Li W. H. (1979): Mathematical Model for Studying Genetic Variation in Terms of Restriction Endonucleases. Proceedings of the National Academy of Sciences, 76, 5269-5273.

Nei, M., Miller, J. C. (1990): A simple method for estimating an average number of nucleotide substitutions within and between populations from restriction site data. Genetics, 125, 873–879.

Perdices, A., Cunha, C., Coelho, M. M. (2004): Phylogenetic structure of *Zacco platypus* (Teleostei, Cyprinidae) populations on the upper and middle Chang Jiang (Yangtze) drainage inferred from cytochrome b sequences. Molecular Phylogenetics and Evolution, 31, 192–203.

Popoola, O. M., Fasakin, E. A., Awopetu, J. I. (2014): Genetic Variability in Culture and Wild Populations of *Clarias gariepinus* (Osteichthyes: Clariidae) using Random Amplified Polymorphic DNA (RAPD) Marker. Croatian Journal of Fisheries, 72, 5–11.

Rognon, X., Guyomard, R. (2003): A large extent of mitochondrial DNA transfer from *Oreochromis aureus* to *O. niloticus* in West Africa. Molecular Ecology, 12, 2, 435-445.

Rozas, J., Sanchez-DelBarrio, J. C., Messeeguer, X., Rozas, R. (2003): DnaSP, DNA polymorphism analysis by the coalescent, and other methods. Bioinformatics, 19: 2496–7.

Sati, J., Kumar, R., Sahoo, P. K., Patiyal, R. S., Ali, S., Barat, A. (2015): Genetic characterization of Golden mahseer (*Tor putitora*) populations using mitochondrial DNA markers. Mitochondria DNA, 26, 1, 68-74.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011): MEGA5: Molecular evolutionary genetics analysis using likelihood, distance, and parsimony methods. Molecular Biology and Evolution, 28, 2731–9.

Ullrich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W. J., Goodman, H. M. (1977): Rat insulin genes: construction of plasmids containing the coding sequences. Science, 196, 4296, 1313-1319.

Vrijenhoek, R. C. (1998): Conservation genetics of freshwater fish. Journal of Fish Biology, 53, 394–412.

Ward, R. D., Woodwark, M., Skinbinski, D. O. F. (1994): A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. Journal of Fish Biology, 44, 213–32.

Yakubu, A., Okunsebor, S. A. (2011): Morphometric differentiation of two Nigerian fish species (*O. niloticus* and *Lates niloticus*) using principal components and discriminant analysis. International Journal of Morphology, 29, 1429-34.