Comparative study of single nucleotide polymorphisms (SNPs) of a candidate growth gene (IGF-I) in Oreochromis niloticus and Sarotherodon melanotheron

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In teleost fishes, the regulation of growth performance by the GH and IGF system also seems to be highly conserved. Adult Oreochromis niloticus (Nile tilapia) could reach up to 60 cm maximum length while Sarotherodon melanotheron (black chin Tilapia) has a maximum length of 28 cm. This study describes the analysis of Single Nucleotide Polymorphism in isolated, amplified, and sequenced DNA from two common Tilapia species (O. niloticus and S. melanotheron) with the aim of identifying genetic variation and single nucleotide polymorphisms (SNPs) in one of the main genes, Insulin like growth Factor-1 (IGF-I), related to growth in the Tilapia species. The extracted DNA from the clipped caudal fins of the Tilapia species samples using Sambrook and Russell’s modified chloroform/isoamyl alcohol DNA extraction method were further amplified in a thermal cycler with designed IGF-I forward and reverse primers of 447 bp which were subsequently sequenced with an automated analyzer. The PCR product was separated on 1.5% ethidium stained agarose gel electrophoresis and the bands revealed on the gel were all of the same length (447 bp). The Sequence alignment revealed a total of five single nucleotide polymorphisms which were detected in the forward reaction at the positions 181, 199, 328, 362 and 369 of the sequences and in the reverse reaction at positions 18, 20, 54, 183 and 201. A total of 138 amino acid sequence was translated from the DNA sequence with variations sequence at positions 1, 3, 4, 61, 67, 121 and 123. These results showed variations among these two fish species which could explain differential growth performance between them.

Key words: Single nucleotide polymorphism (SNP), Oreochromis niloticus (ON), Sarotherodon melanotheron (SM), Tilapia and insulin-like growth factor (IGF).

INTRODUCTION

Single nucleotide polymorphism (SNP) markers are the method of choice for genetic analyses including diversity and quantitative trait loci studies (Thu et al., 2017). Determination of the genetic variability was described by Lupchinski Jr et al. (2011) as an essential step for the implementation of genetic improvement programs that
are focused on the selection of faster growing fish with lower feed conversion rates and resistant to diseases. Studies of genetic diversity at DNA level represents in expansion field in aquaculture aimed at finding out those DNA variations associated with productive phenotypes, so as to use them as tools for assisting the offspring selection at early stage and possibly predict their performance (Na-Nakorn and Moekim, 2009). This strategy is known as gene-assisted selection (GAS) (De-Santis and Jerry, 2007). Growth performance is often used as an indicator of the status of individuals and populations in culture and the wild, and therefore, major effort has been applied towards garnering a more comprehensive understanding of how multiple components of the GH (growth hormone) and IGF (insulin-like growth factor) system interact to control growth and metabolism (Picha et al., 2008; Beckman, 2011). To improve growth and growth efficiency in aquaculture, an advanced understanding of the physiological mechanisms that regulate growth in fishes are needed. The growth hormone/insulin-like growth factor (GH/IGF) in the endocrine axis regulates growth in all vertebrates, including fishes as described by Davis et al. (2008).

Tilapia is the common name for nearly a hundred species of cichlids from the tilapinna cichlid tribe. Tilapia are mainly freshwater fish inhabiting shallow streams, ponds, rivers and lakes and less commonly found living in brackish water. The survey carried out by Oguntade et al. (2014) shows that some fish species including Tilapia are fast disappearing in Nigerian water bodies such as Brass and Nun River of Niger Delta. In 2017, according to FAO statistics, Nile tilapia (Oreochromis niloticus) culture alone was ranked fourth among the most cultivated in the world, in terms of both production and value with a total aquaculture production of 4.1 million tonnes (FAO, 2019). The other top four species were silver carp, grass carp, common carp and other Cyprinids (FAO, 2019). Nile tilapia represents approximately 86% of total global tilapia production (FAO, 2019). In 2017, it is anticipated that global Nile Tilapia production will reach nearly 4 million tonnes (FAO, 2017). Adult Oreochromis niloticus (Nile tilapia) reach up to 60 cm maximum length while Sarotherodon melanotheron (black chin Tilapia) has a maximum length of 28 cm (Olaosebikan and Raji, 1998) when subjected to the same environmental condition. This justifies the higher value and demand for Nile tilapia, hence higher production of other Tilapia species is needed to meet the demand for Tilapia. Black chin Tilapia on the other hand thrive well in high salinity region but are constrained by their growth and as such do not meet market value. Since IGF-I also regulate growth in fishes; the need to study its variation in the two Tilapia species arises with the aim of identifying a possible growth factor that will promote a higher production of S. melanotheron from saline environment to complement the production of O. niloticus from fresh water bodies to meet global tilapia demand. This would be achieved by detecting the genetic variations and single nucleotide polymorphisms (SNPs) in one of the main growth genes, Insulin like growth Factor-1 (IGF-I) in two Tilapia species.

MATERIALS AND METHODS

Fish sample collection and identification

Ten (10) live female tilapia fishes comprising five Oreochromis niloticus (ON1, ON2, ON3, ON4 and ON5) and five Sarotherodon melanotheron (SM1, SM2, SM3, SM4 and SM5 ) were obtained from Nigerian Institute for Oceanography and Marine Research (NIOMR), Badore outstation (latitude 6°25′60″N and longitude 3°510″E), and Oluwo market sourced from Epe river, Epe (latitude 6°35′2″N and longitude 3°59′0″E), Lagos state, Nigeria respectively. The samples of caudal fins were clipped and transferred into ten different plain 10 mL sterile tubes each containing 4 mL absolute ethanol.

Fish samples were identified from description checklist and identification keys (FAO, 1996; Froese and Pauly, 2003; Uyeno and Fuji, 1984).

DNA extraction and amplification

DNA was isolated from the caudal fin tissue of the ten sampled fish using modified chlorophenol/isoamyl/alcohol protocol according to Sambrook and Russell (2001) on bench at biotechnology laboratory of NIOMR, Badore outstation, Nigeria. The integrity of the DNA was checked on 1% ethidium bromide stained agarose gel electrophoresis and the isolate was stored at -20°C prior PCR amplification.

The PCR amplification was run with the specific primers (IGF-I forward- 5'-CTTGGACGAGTAGGAGCGAAATG-3' and IGF-I reverse- 3'-GAAATACAGCAAGCGATAAGAA-5') of 447 bp designed to amplify coded regions (exons) of the IGF-I gene sequences (IGF-I, GenBank accession AF033797) which was re-sequenced and used. The DNA amplification was carried out by polymerase chain reaction (PCR) in a with 20 ng of genomic DNA using Thu et al. (2017) protocol, 20 ul reactions containing 0.2 μM of each primer, 200 uM of dNTPs, 50 mM KCl, 10 mM Tris HCL (pH 8.3), 1.5 mM MgCl₂ and 0.5 units of Taq DNA polymerase with Eppendorf thermocycler with an amplification profile of initial denaturation at 95°C for 10 min, followed by 35 cycles with 95°C for 30s, annealing temperature at 60°C for 45s, extension at 72°C for 45s and final extension at 72°C for 5 min. The product was checked on 1.5% agarose gel electrophoresis at 70v for 1.5 h in 1x TBE buffer and the gel was stained with ethidium bromide for visualization through Fisher Scientific UV transilluminator.

DNA sequencing

Purified PCR products from the amplification of the ten Tilapia

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fishes, *O. niloticus* (5) and *S. melanotheron* (5) were bidirectional sequenced in an automatic sequencer (ABI 3500XL Genetic Analyzer).

Nucleotide sequences obtained were edited and aligned using clustal O (version 1.2.4) multiple sequence alignment software, the Single nucleotide Polymorphisms (SNPs) were discovered by visual analysis and dendogram was also created while translation of the DNA sequence of each species was done using biolign alignment software (version 4.0.6.2).

**RESULTS AND DISCUSSION**

**DNA extraction and amplification**

The IGF-I amplified PCR product of the extracted DNA run on 1.5% ethidium bromide stained agarose gel demonstrated that IGF-I genes had the same bands which demonstrated equal fragment length of the ten Tilapia fishes, *O. niloticus* (5) and *S. melanotheron* (5) as shown on Plate 1, where M is the known 50 bp- 10 kb DNA ladder.

**DNA sequencing and analyses**

The sequence alignment generated for the forward and reverse primers of IGF-I of the ten tilapia fish (5 *Oreochromis niloticus* and 5 *Sarotherodon melanotheron*) were shown in Figures 1 and 2 respectively while Tables 1 and 2 show the five single nucleotide polymorphisms (SNPs) detected at the positions 181(T/C), 199(T/C), 328(C/G), 362(C/A), and 369(A/C) of the forward reaction sequence and those detected in the reverse reaction sequence at positions 18(T/A), 20(T/G), 54(C/G), 183(G/A), and 201(G/A) respectively.

The dendogram revealed lower similarities between SM1-SM5 and ON1-ON5 and higher similarities among SM1-SM5 and among ON1-ON5 (Figure 3). The lower similarity between SM and ON might imply a high genetic variation and could be due to the fact that they are different species and do not have common ancestry. This finding is on the contrary with the report of Usman et al. (2013) who obtained a high similarity coefficient of 78% between *T. guineensis* and *S. melanotheron* from the wild.

A total of 138 amino acid sequence was translated from the DNA sequence of *O. niloticus* and *S. melanotheron* as shown in Figures 4 and 5. The alignment of these sequences revealed seven (7) Variations at positions 1, 3, 4, 61, 67, 121 and 123 as shown in Figure 6.

The variations observed are as follows; position 1 R (arginine) in SM to V (valine) in ON, position 61 Q (glutamine) in SM to *(stop codon)* in ON (Table 3).

This study described the use of Single Nucleotide Polymorphism (SNP) markers to validate genetic variation in a candidate growth gene (IGF-I) in *O. niloticus* and *S. melanotheron*. The DNA sequences gotten from primer used in this study was about 447 bp in agreement with the result obtained by Cuevas-Rodríguez et al. (2016). Among the growth genes, IGF-I is said to contribute in a variety of physiological processes, such as growth, metabolism, reproduction and osmoregulation (Reinecke et al., 2005) in teleosts. Thus variation of IGF-I might be a good reason for the growth difference between *O. niloticus* and *S. melanotheron*.

It was summarized that there are indeed variations in the candidate growth gene, IGF-I of the two Tilapia species and this might be a reason for the significant difference in their growth rate. Thus improved varieties of
CLUSTAL O(1.2.4) multiple sequence alignment

SM1_IGF-1F     ----------------AGTCTGTGTATGTAGATAAATGTGAGGGATTTTCTCTCTAATC 44
SM3_IGF-1F     ---------------------------------- 53
SM4_IGF-1F     --CTTCTTCTGTATGAGGGATTTTCTCTCTAATC 58
SM5_IGF-1F     -CGCTTTCTGTATGAGGGATTTTCTCTCTAATC 59
SM2_IGF-1F     CTTCTTCTGTATGAGGGATTTTCTCTCTAATC 60
ON1_IGF-1F     GTTCTTCTGTATGAGGGATTTTCTCTCTAATC 60
ON4_IGF-1F     --CGGCTTTCTGTATGAGGGATTTTCTCTCTAATC 58
ON2_IGF-1F     ---------------------------------- 47
ON3_IGF-1F     -CTTCTTCTGTATGAGGGATTTTCTCTCTAATC 59
ON5_IGF-1F     -GTTCTTCTGTATGAGGGATTTTCTCTCTAATC 59

SM1_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 104
SM3_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 113
SM4_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 118
SM5_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 119
SM2_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 120
ON1_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 119
ON4_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 118
ON2_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 107
ON3_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 119
ON5_IGF-1F     CGCTCTTCTGTATGAGGGATTTTCTCTCTAATC 119

SM1_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 164
SM3_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 173
SM4_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 178
SM5_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 179
SM2_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 180
ON1_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 180
ON4_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 178
ON2_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 167
ON3_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 179
ON5_IGF-1F     GAATATTGGATGAGGGATTTTCTCTCTAATC 179

SM1_IGF-1F     CAAACAGTTTCTTCTTCTCTCTAATC 224
SM3_IGF-1F     CAAACAGTTTCTTCTTCTCTCTAATC 233
SM4_IGF-1F     CAAACAGTTTCTTCTTCTCTCTAATC 238
SM5_IGF-1F     CAAACAGTTTCTTCTTCTCTCTAATC 239
SM2_IGF-1F     CAAACAGTTTCTTCTTCTCTCTAATC 240
ON1_IGF-1F     TAAACAGTTTCTTCTTCTCTCTAATC 240
ON4_IGF-1F     TAAACAGTTTCTTCTTCTCTCTAATC 238
ON2_IGF-1F     TAAACAGTTTCTTCTTCTCTCTAATC 227
ON3_IGF-1F     TAAACAGTTTCTTCTTCTCTCTAATC 239
ON5_IGF-1F     TAAACAGTTTCTTCTTCTCTCTAATC 239

SM1_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 284
SM3_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 293
SM4_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 298
SM5_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 299
SM2_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 300
ON1_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 300
ON4_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 298
ON2_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 287
ON3_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 299
ON5_IGF-1F     TTTCTTTCTTCTTCTTCTCTCTAATC 299

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Figure 1. Alignment of the forward reaction of the IGF-1 of the ten tilapia fish (5 *O. niloticus* and 5 *S. melanotheron*). *Mean similarities among the sequence bases of the ten fishes.

| SNP position | ON | SM |
|--------------|----|----|
| ON1_IGF-1    | ACTATACATTACACTTTGATTCTCTCGATTGCTCACTATTGGGACAGACAGATCTCGCT | 358 |
| ON2_IGF-1    | ACTATACATTACACTTTGATTCTCTCGATTGCTCACTATTGGGACAGACAGATCTCGCT | 358 |
| ON3_IGF-1    | ACTATACATTACACTTTGATTCTCTCGATTGCTCACTATTGGGACAGACAGATCTCGCT | 358 |
| ON4_IGF-1    | ACTATACATTACACTTTGATTCTCTCGATTGCTCACTATTGGGACAGACAGATCTCGCT | 358 |
| ON5_IGF-1    | ACTATACATTACACTTTGATTCTCTCGATTGCTCACTATTGGGACAGACAGATCTCGCT | 358 |

Table 1. SNPs detected between the forward nucleotide sequence reaction of the ten Tilapia fishes (*Oreochromis niloticus* and *Sarotherodon melanotheron*).

| SNP position | ON | SM |
|--------------|----|----|
| 181          | T  | C  |
| 199          | T  | C  |
| 328          | C  | G  |
| 362          | C  | A  |
| 369          | A  | C  |
CLUSTAL O(1.2.4) multiple sequence alignment

SM2-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

SM1-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

SM5-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

SM3-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

SM4-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

ON4-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

ON3-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

ON1-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

ON2-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

ON5-IGF-1R: ATCAAGGTTGATATATTAGTATTGCTCAAAGAAAGATCGTAAGTTACTCTTGAGAAGCAGACATCAACA

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SM2-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

SM1-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

SM5-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

SM3-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

SM4-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

ON4-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

ON3-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

ON1-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

ON2-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

ON5-IGF-1R: TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC

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SM2-IGF-1R: CGCGAAATGACCTTTGTGGAACTTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

SM1-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

SM5-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

SM3-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

SM4-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

ON4-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

ON3-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

ON1-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

ON2-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

ON5-IGF-1R: CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG

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Figure 2. Alignment of the Reverse Reaction of the IGF-1 of the ten tilapia fish (5 Oreochromis niloticus and 5 Sarotherodon melanotheron). *Mean similarities among the sequence bases of the ten fishes.

Table 2. SNPs detected between the reverse nucleotide sequence reaction of the two Tilapia species (O. niloticus and S. melanotheron).

| SNP position | SM   | ON   |
|--------------|------|------|
| 81           | T    | A    |
| 20           | T    | G    |
| 54           | C    | G    |
| 183          | G    | A    |
| 201          | G    | A    |

Table 3. Variations detected between the amino acid sequence of the two Tilapia species (O. niloticus and S. melanotheron).

| Sequence position | SM | ON |
|-------------------|----|----|
| 1                 | R  | V  |
| 3                 | L  | S  |
| 4                 | V  | F  |
| 61                | Q  | *  |
| 67                | P  | S  |
| 121               | N  | T  |
| 123               | N  | K  |

*S*Mean stop codon.

S. melanotheron with bigger sizes might be achievable. Also, regions of higher salinity where S. melanotheron strive well can be encouraged to grow the improved varieties and the demand for S. melanotheron increases.
Figure 3. Dendogram (phylogenetic tree) of the DNA sequence from IGF-1 for the ten Tilapia (5 *O. niloticus* and 5 *S. melanotheron*).

Figure 4. Translation of DNA sequence of IGF-1 of *O. niloticus* (ON) to amino acid. *Mean stop codon.
Figure 5. Translation of DNA sequence of IGF-1 of *S. melanotheron* (SM) to amino acid. *Mean stop codon.

![DNA sequence translation](image)

Figure 6. Amino acid sequence alignment from the translation of the DNA sequence of IGF-1 of *Oreochromis niloticus* (ON) and *Sarotherodon melanotheron* (SM). * Mean stop codon.

![Amino acid sequence alignment](image)
This study serves as baseline information in selective breeding whereby the amino acids present in the IGF-I of *O. niloticus* may be fed orally to *S. melanotheron* by adding them to their feed while growing.

**CONFLICTS OF INTEREST**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

The authors are very grateful to the management team of NIOMR for granting the time and space for the project. The efforts of other colleagues at the department of Biotechnology, Nigerian Institute for oceanography and Marine Research, Lagos (NIOMR) for their wonderful contribution towards the laboratory analysis are highly appreciated.

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