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Sequencing and Sequence Analysis of Growth Hormone Type 1 (GH1) Gene Analogue in the Salmo Trutta Caspius

[1]Abolhasan Rezaei and [2]Sheyda Akhshabi
[1]Department of Genetics-School of Basic Science, Tonekabon Branch,
Islamic Azad University, Tonekabon, Iran
[2]Young Researchers Club, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran

Abstract: The Growth hormone gene is more important in the regulator of metabolism, osmoregulation, reproduction and skeletal growth in Livestock. Specially, in the *Salmonids*. Furthermore, growth hormone gene can be used as the fusion of phylogenetics, population genetics and allows assessment of the impact of historical events on the current relationships among different populations. Growth hormone gene in the *Atlantic Salmon* (*Salmo salar*) has full length of almost 3.5 kb at different reports in the Gene Bank. For studying of growth hormone gene in the *Salmo trutta caspius*, was extracted DNA genomics from bloods and the muscles of *salmons*, in related to, first we designed three pairs of the primers from the full length of the growth hormone gene. These primers can be synthesis different fragments which contain five introns and six exons in the full length of GH1. After doing PCR, PCR products were purified using the Gel extraction SPIN-50 (RKT33) kit, then sequence products, analyzed by the NCBI Network system. Sequence fragments were shown there is a high homology with other GH gene *salmons* in the exon and intron fragments. Furthermore, a complete sequence of GH gene in the *Salmo trutta caspius* deposited in the GeneBank, accession number, JN241634.1. That is including 2041 by a length. In this research we had two aims, first, the study of amount variation in the between *salmo trutta caspius* species with *Atlantic salmon* and second aim, the study amount of variation *Salmo trutta caspius* with other salmons regarding to the growth hormone gene.

Key words: Growth hormone gene, *Salmo trutta caspius*

INTRODUCTION

In recent decades, researchers have introduced many structural genes for development of aquaculture, e.g. different growth hormone genes (GH) (Uzbekova *et al*., 2000). Growth hormone gene in mammals, is encoded by a single-copy gene containing nine exons (Maj *et al*., 2004a, b), while in fish, this gene contains an additional exon and is presented as a double copy, GH-I and -II (Almuly *et al*., 2000; Yue *et al*., 2001; Yue and Orban, 2002; Almuly *et al*., 2005). *Salmonids* GH I and II are both actively transcribed, but their expression is not equally partitioned exhibiting some tissue-specificity (Saera-Vila *et al*., 2005). There are six exons and five introns in different species of *salmonids*, in related to, this is different size length in the introns and exons between and within different species of *salmonids* (Forbes *et al*., 1994). For example, in the Pacific salmon there are two largest introns which named intron C and intron D, which average 805bp for GH1 C and 409bp for intron GH2C and 1010bp for intron GH1D and 1048bp for intron GH2D. Therefore, by the analyze of phylogenetic regarding the length of intron and exon has been used for examination of relationships by *Salmo trutta Caspius*. Ryynanen and Primmer (2004), where shown, that duplicated genes (GH I and II) can diverge in function as a result of mutations in regulatory elements, also they were shown GH I and II have provided by the ancestral locus around 25-50 million years ago. The duplicated GH genes may functions provided by the ancestral locus (Agellon *et al*., 1988). The duplicates may experience differences in selective pressures as a result of this divergence that could affect their usefulness for phylogenetic analysis . At all of the reports of GH genes in NCBI Network has almost 3.5 kb about the full length of the growth hormone gene and 210 amino acids. (For ex. Accession number:AY614005). We have previously isolated and sequenced the DNA of growth hormone gene type 1 from *Salmo trutta caspius*, (Rezaei *et al*., 2011a, b). Also, this gene has deposited in the NCBI network, accession number, JN241634.1. In order to analyze this gene and to determine in its relationship with other salmons growth hormone gene, we have found together a high homology within and between species in the exon's lengths, but there is the variation in the length of the introns, so we can report that *Salmo trutta caspius origin* from *Atlantic salmon*. However, the goals of this research were to first, provide information from the full length of GH1 gene in *Salmo trutta caspius*, second, for to test segregation of the variants in the within salmo

Corresponding Author: Abolhasan Rezaei, Department of Genetics-School of Basic Science, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran

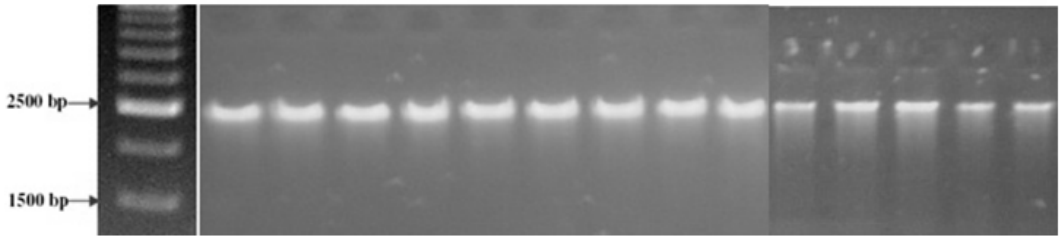

Fig. 1: PCR amplification of Growth hormone gene of *Salmo trutta caspius* including 2048 bp. PCR Product was loaded on 1% agarose gel

Table 1: Primers used in this study

Primer	Sequence (5´ TO 3´)	Product size
Fwd primer (SsGH1):	ACATACTCAACCGACCACCGCACTTTCAAG	910
Rev primer (SsGH2):	GTGACAGGTCCACTCTGCTATTCA	
Fwd primer (SsGH3):	GTAAATAGGGAATCTCAAGCTGT	312
Rev primer (SSGH4):	CTCAAATACTTCTAGTAAGTTGA	
Fwd primer (SsGH5):	CATCACTAATATTGACTATATCAG	819
Rev primer (SsGH6):	CAGATTAGGCCTTGCCCTGCACTGA	
Sequence primer:	ATCTGGTAGAGCCTGACTCCA	

trutta caspius species, third, for the relationship between Salmo trutta caspius and other species *salmonids* by the via polymorphism of the GH1 gene, fourth, for the relationship between *Salmo trutta caspius* and other nonsalmonids, for the future transfer and cloning of the GH genes.

MATERIALS AND METHODS

Samples: The *Salmo trutta caspius* populations were taken from two rivers (river of Dohezar and river of Sardabroud in North of Iran).These fishes had almost 3 years age old. They were anaesthetized with MS2220 and extracted of blood from the caudal vein. Blood samples (2-5 mL) were removed from the fish via caudal puncture (G.18 Needle) using a heparinized syringe (as an anticoagulant for blood sampling) after using MS222, (1:10,000) as an anesthetic to minimize stress.

DNA extraction and PCR amplification: Total Genomic DNA isolated from the bloods, and muscles according to the procedure simplify the phenol-chloroform method (Sambrook *et al*., 1989). 400 μL of lysis buffer (100 mM Tris-HC1 pH 8.5, 5 mM EDTA, 0.2% SDS, 200 mM NaCl) and 20 μL of proteinase K and 400 μL phenols were added to the lyses blood sample and incubated at 37ºC overnight. The DNA was precipitated by adding one volume of ethanol 75% in 3 times and was dissolved in 50 μL of TE buffer (10 mM Tris-HC1, 1 mM EDTA, pH 8.0).PCR reactions contained: 1 μL template DNA, 2 μl forward primer (100 ng/μL), 2 μL reverse primer (100 ng/μL), 2 μL dNTP mix (2.5mM each), 5 μL 10X ChromTaq Assay buffer, 0.5 μL ChromTaq enzyme (3U/ μL), Water 37.5 μL, in a total volume, 50 μL. 94º of 5 min, 35 cycles of 94ºC 30 sec., 55ºC 30 sec., and 72ºC 1 min. 2 to 10 μL of each PCR reaction were run on 1% agarose gels in TAE buffer containing ethidium bromide. 1 μL 500bp, DNA ladder (Gibco-BRL) was used as a size standard.

The GH gene sequencing: DNA was prepared for sequencing by running 20 μL PCR reaction product in a 1% agarose gel and excising the desired bands. DNA genomics was isolated as bands on the gel electrophoresis. If necessary to produce enough templates, single alleles were re-amplified from a gel slice soaked in 100 μL TE and frozen overnight at -40ºC. Template DNA was purified from gel slices using GEL EXTRACTION SPIN-50 (RKT33). Automated sequencing was done by dye termination PCR cycle sequencing, using the original amplification primers, in an Applied Biosystems Incorporated 373 automated DNA sequencer. An internal sequencing primer used for some sample, that including:

ATCTGGTAGAGCCTGACTCCA

RESULTS AMD DISCUSSION

In the study, we amplified almost 2054 nucleotides by using the polymorphism chain reaction technique (Fig. 1). In the presence on the nucleotides primers including, SsGH1-SsGH 2- SsGH3-Ss GH4- SsGH5-SsGH6, (Table 1). DNA expected length was amplified from extracted blood samples from the *Salmo trutta caspius* GH-I gene. These primers were also amplified fragments, 910, 310 and 819 bp. Since there is not any reported about the sequence of the GH gene of *Salmo trutta caspius* in Gene Bank. However, for designing primers, we aligned from first to end of some sequences of GH gene of *Salmo salar* from the NCBI Network

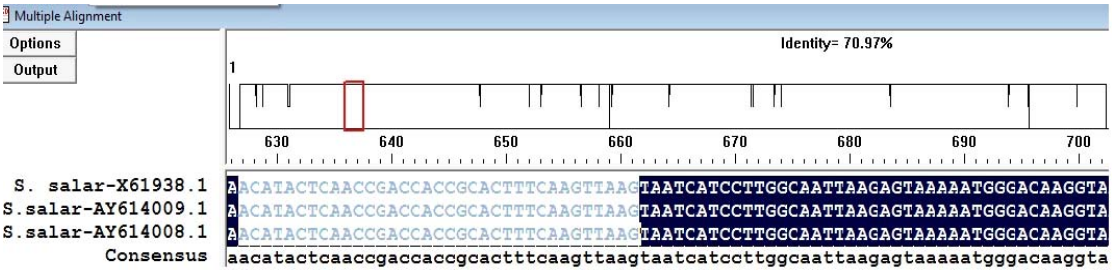

Fig. 2: The Sequence comparison for growth hormone gene from some *Salmon* sequences for designing forward primer. Shades denote high homology between sequences

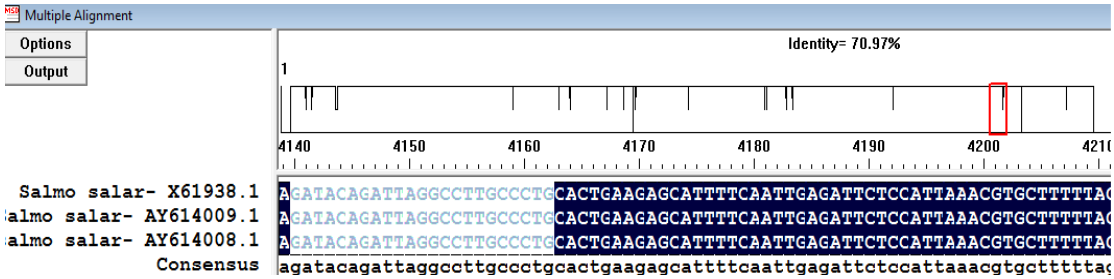

Fig. 3: The sequence comparison for growth hormone gene from some salmon sequences for designing the reverse primers. Hence, we used three sequences from salmons. Similar sequences from nucleotide of 4140 to 4162, are shown; shades denote the high homology between sequences

system (Gene Bank) Hence. We used three sequences to 4162, are shown; (Fig. 2 and 3), because we had expected that *salmo trutta caspius* has been high homology with other salmons, specially, *Salmo salar* and *Salmo trutta*. Moreover, *Salmo trutta* and *Salmo salar* fish had been high morphology with Salmo trutta caspius. For getting the Growth hormone gene contain exon and intron, were synthesized from *Salmo trutta caspius* (Fig. 4).

The Growth hormone gene in Salmonids is represented by duplicated. Non-allelic isoforms designated as GH1 and GH2 (Johonsson and Einarsson, 1993), which diverged at least 25-30 million years (MY) ago (Gross *et al*., 1996). The full length of GH gene sequenced in different species of salmons, including, *Atlantic salmon*, *chinook salmon*, *coho salmon*, *Salmo trutta* and its similar species that reported by NCBI Network system. In the present study, we have sequenced five distinct fragments GH gene from *Salmo trutta caspius* samples. The homology analysis based on the genomic sequences identities further indicated that the GH genes had high homology to the GH gene's salmon species. By the phylogenetic analysis and homology analysis, all the results indicated that the newly DNA encoded two distinct types of GH genes in salmons. Interestingly, recent research in Japanese medaka (Oryzias latipes) suggested that most of the genes reported as GH receptor from other fish species (especially the non-salmonid fishes) might be, at least phylogenetically, receptors for somatolactin (SLRs) (Fukamachi *et al*., 2005). So in the present study, we compared between sequences of GH genes with some fish GH genes, and then we found that had high identity of almost 90% to the similar sequence features with eleven sequences of GH gene in salmonids and in other fish (Fig. 5) .

In the present study, we noticed that the length of the introns detected by the sequencer Chromous Company. In the analyze of the phylogenetic tree showed that GH genes in teleost fish could be diverted into two clades. The GHs of non salmonid fishes belonged to GH1 or GH2 clade respectively. On the other hand, the GH of all salmonids, discussed, such as *coho salmon*, *Atlantic salmon* and *rainbow trout* belonged to same clade. Therefore, it suggested that the GH genes reported in salmonids should be the different isoforms or spliced variants of GH2 actually. Evolution by gene duplication the old hypothesis had been proven and accepted. Gene's duplications might be responsible for the functional diversification of genes, the creation of gene families and the increased genomic (Ohno, 1970). The vertebrates got evolution by the Whole Genome Duplications (WGD), while fish (especially the teleost) had undergone an additional WGD (Wittbrodt *et al*., 1998). Therefore, more than one GH genes were maintained by selection through the radiation in the teleosts. On the other hand, ancestral existence and consequent loss of GH genes in the higher vertebrates seem likely (Fukamachi *et al*., 2005). So the concurrence of two GHs might possibly exist in some

Sequence data obtained from Salmo trutta caspius

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1    CAAAAAATCA TCCCTTGCGC AATTAAGAGT AAAAATGGGA CAAGGTAAGC CTGCTTTTTC
61   TGTCTATTTC TTTTTTTAGT GGGAAGTCAG TGTACCATTT AGTACAGTTT AACTTACACA
121  TTTAATCACT GAGGCAGGGG CCAACACGGC AGAGAAAAGT GAACAAGTAT TCTACTACTA
181  TGAGGTTATA ATCTATTGAC ACAGAACCAC CTGCTTTAAC AACCTAACTA TGTGATCTAT
241  AACATTTACA TTTGAGTCAT TTAGCAGACA CTCTTATCCA AAGCGACTTA CAGGAGCCAT
301  TAGGGTTAAG TGCCTTGCTC AAGGGCACAT CGACAGATTT CTCACCTAGT CAGCTCAGGG
361  ATTGAAACCG GTAACCTTTC AATTACTTAC CCAACGCTCT TAACCGCTGG GCTATTGGTG
421  TACAATGGCT GAGAATATCT AACTAATGTA TCTCACCATA ATTCGACTTA CTCGTTTTAT
481  ACATTTCTTA TTTTATTTAA TCTCTCTTTT AGTGTTTCTG CTGATGCCAG TCTTACTGGT
541  CAGTTGTTTT CTGAGCCAAG GGGCAGCGAT GGAAAACCAA CGGCTCTTCA ACATCGCGGT
601  GAACCGGGTG CAACATCTCC ACCTAATGGC TCAGAAGATG TTCAATGACT TTGTAAGACA
661  GCTTTTGAAT CTTCTTTTGA CATATCAAAT AATGTATTAA TGATTGTTCT TCTTCTTGTA
721  GACAGTATCC TCTTTACACA ACCCCTCGCG GCTAAAAAAA AACAACAGAA AATCTCTCTC
781  CCTTTCTTTG TGATTTTGTG CAGGAAGGCA CCCTGTTGCC TGATGAACGC AGACAGCTGA
841  ACAAGATATT CCTGCTGGAC TTCTGTAACT CTGACTCCAT CGTGAGCCCA ATCGACAAGC
901  TTGAGACTCA GAAGAGTTCA GTAAGTAACC TGGCTGAGAC AATACGCATG GACGCCCTTA
961  AAACCCCTTA AATTTGGCGG GTTGGTGTAA ATAGGGAATC TCAAGCTGTA CAATACAACG
1021 CAACTTCATT TTCCAATAAT CTGTGGTTTC TCTACACACA CAGGTCCTGA AGCTGCTCCA
1081 TATCTCTTTC CGTCTGATTG AATCCTGGGA GTACCCTAGC CAGACCCTGA CCATCTCCAA
1141 CAGCCTAATG GTCAGAAACT CCAACCAGAT CTCTGAGAAG CTCAGCGACC TCAAAGTGGG
1201 CATCAACCTG CTCATCAAGG TAAAGAAAGG AGGGAGAACA ATGACCATTT GTGGTGCCAC
1261 ACTTTGTGCA CTGTAAACCC CAAGGCATTT TTAACTCAAA TACTTCTAGT AAGTTGAAGT
1321 TGTGCTATAT CAGTAACACC CCATTCAATG ACTGAATATC GGCCCATTCA AGGATATTTA
1381 TGCATGTTTC TTTTGCCGTG TGTGCTTTCA GAAAGGCCCA ATAAACAAAT ATTGATATGC
1441 ACACATCCAT GCATCTCTCT CTGTCTCCCA CAGGGGAGCC AGGATGGCGT ACTGAGCCTG
1501 GATGACAATG ACTCTCAGCA GCTGCCCCCC TACGGGAACT ACTACCAGAA CCTGGGGGGC
1561 GACGGCAACG TCAGGAGGAA CTACGAGTTG TTGGCCTGCT TCAAGAAGGA CATGCACAAG
1621 GTGCAAAACC ATGTTGCCTT CAATTTCATG TACCTTCCTA TATTTTTTAC AGTGCGTTGT
1681 TTTTTTGTGC TCTCTATTGC AAAGTATCTT TGGGTCTTTA ACCCATATAT TATTACTATT
1741 ATTGTTCATT GATCAAGACT GTTCTCGAGA AAGGTCTAGT GACCTAGAAC ACTCACATTA
1801 AAATGTGTCA ACTATAACCC ATTCTTCTAT TTTTTCCCCC CAAGGTCGAG ACCTACCTGA
1861 CCGTCGCCAA GTGCAGGAAG TCACTGGAGG CCAACTGCAC TCTGTAGACG TGGGCTGGAG
1921 AGGCAGCCAG CAAGAGCCTG TCTCCAGGGT TCGGTTTCCC AGATACAGAT TAGGCCTTGC
1981 CCTGCACTGA ACAGCATTTT CGATTGAGAT TCTCCATTAA AACATGCTTC TTTTGTTGTG
2041 GAGTAAAG
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Fig. 4: The nucleotide sequence of the growth hormone gene in the *Salmo trutta caspius* GH gene. There are six exons in the reference sequence (as *Salmo salar*). All six exons are highlighted in the above sequence data obtained from *Salmo trutta caspius* growth hormone gene. Red highlighted are exons, and Black highlighted are introns

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
JN241634.1	Salmo trutta caspius growth hormone gene, complete cds	3783	3783	100%	0.0	100%
EU621898.1	Salmo salar clone 11F04_73D15 interferon alpha 1-like gene, complete sequenc	1513	3313	97%	0.0	97%
AY614010.1	Salmo salar isolate Ss11_GH1 growth hormone I gene, complete cds	1513	3233	97%	0.0	97%
AY614009.1	Salmo salar isolate Ss10_GH1 growth hormone I gene, complete cds	1513	3233	97%	0.0	97%
AY614008.1	Salmo salar isolate Ss9_GH1 growth hormone I gene, complete cds	1513	3233	97%	0.0	97%
AY614007.1	Salmo salar isolate Ss8_GH1 growth hormone I gene, complete cds	1513	3225	97%	0.0	96%
AY614006.1	Salmo salar isolate Ss7_GH1 growth hormone I gene, complete cds	1513	3233	97%	0.0	97%
AY614005.1	Salmo salar isolate Ss6_GH1 growth hormone I gene, complete cds	1513	3234	97%	0.0	97%
AY614004.1	Salmo salar isolate Ss5_GH1 growth hormone I gene, complete cds	1513	3233	97%	0.0	97%
AY614003.1	Salmo salar isolate Ss3_GH1 growth hormone I gene, complete cds	1513	3234	97%	0.0	97%
AY614002.1	Salmo salar isolate Ss2_GH1 growth hormone I gene, complete cds	1513	3223	97%	0.0	96%
X61938.1	Salmo salar gene for growth hormone I	1506	3234	97%	0.0	97%

Fig. 5: Sequence analysis of the GH gene in *Salmo trutta caspius* with sequence's other salmonids in Gene Bank. Scores denote conserved nucleotides (97%)

teleost species. Additionally, the former reports in GHs of teleost species suggested that GHR2 might be the retained form through the evolution of the teleost species (Fukada *et al*., 2004; Tse *et al*., 2003; Very *et al*., 2005). However, whether the GH1 existed in salmonids needed more evidence and further reassessments.

Sequencing and characterization introns and exons of GH gene in the salmo trutta caspius: We used the polymerase chain reaction (PCR) to amplify variable sections (introns and exons) of the growth hormone in *Salmo trutta caspius*. The *Salmo trutta caspius* had five DNA length variants for introns and six length exons the

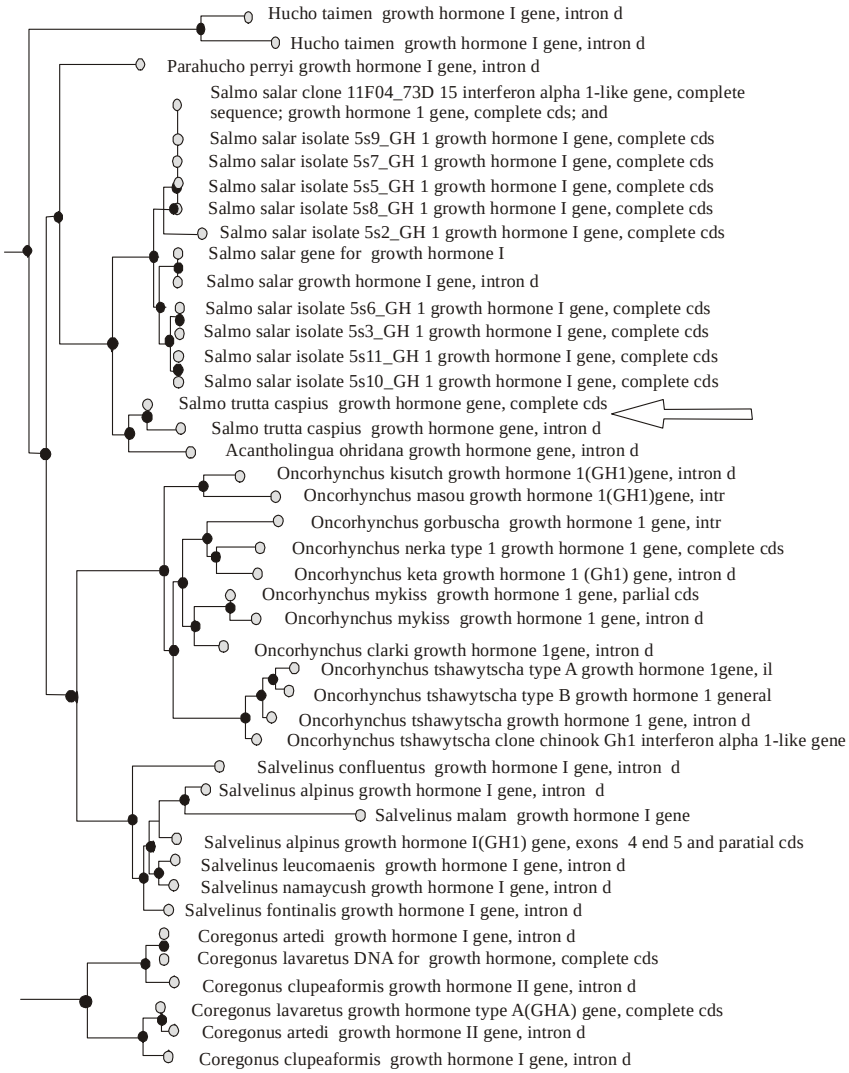
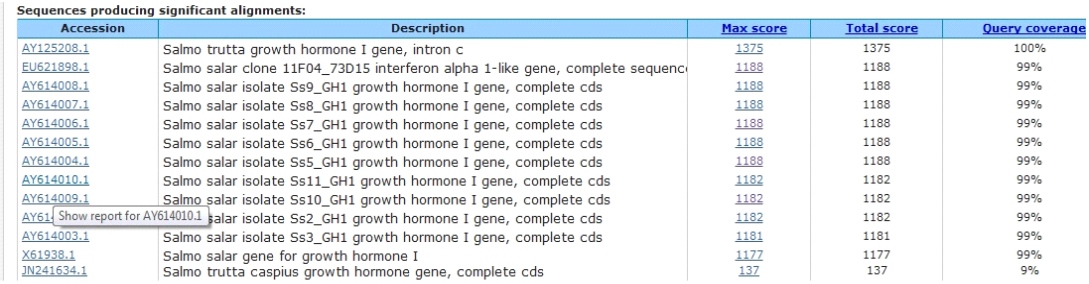


Fig. 6: Phylogenetic relationships of bony fish species based on the GeneBank NCBI network system. The horizontal arrow indicate were ancient between *Salmo trutta caspius* and *Salmo trutta* (*Brown trout*) , also, other bony fishes. Specially other salmons were from an ancient group, that had studied by growth hormone gene

Accession	Description	Max score	Total score	Query coverage
AY125204.1	Salmo trutta growth hormone I gene, intron d	2025	2025	100%
AY614005.1	Salmo salar isolate Ss6_GH1 growth hormone I gene, complete cds	1740	1740	100%
AY614003.1	Salmo salar isolate Ss3_GH1 growth hormone I gene, complete cds	1740	1740	100%
AY614010.1	Salmo salar isolate Ss11_GH1 growth hormone I gene, complete cds	1738	1738	100%
AY614009.1	Salmo salar isolate Ss10_GH1 growth hormone I gene, complete cds	1738	1738	100%
AY614007.1	Salmo salar isolate Ss8_GH1 growth hormone I gene, complete cds	1735	1735	100%
EU621898.1	Salmo salar clone 11F04_73D15 interferon alpha 1-like gene, complete sequence	1733	1733	100%
AY614008.1	Salmo salar isolate Ss9_GH1 growth hormone I gene, complete cds	1733	1733	100%
AY614006.1	Salmo salar isolate Ss7_GH1 growth hormone I gene, complete cds	1733	1733	100%
AY614004.1	Salmo salar isolate Ss5_GH1 growth hormone I gene, complete cds	1733	1733	100%
X61938.1	Salmo salar gene for growth hormone I	1727	1727	100%
JN241634.1	Salmo trutta caspius growth hormone gene, complete cds	233	417	23%

Fig. 7: Sequence analysis of the Growth hormone gene in *Salmo trutta* growth hormone 1 gene, intron d with sequence's other salmonids in Gene Bank. Scores denote conserved nucleotides.(100%) and *Salmo trutta caspius (*23%)



Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage
AY125208.1	Salmo trutta growth hormone I gene, intron c	1375	1375	100%
EU621898.1	Salmo salar clone 11F04_73D15 interferon alpha 1-like gene, complete sequenc	1188	1188	99%
AY614008.1	Salmo salar isolate Ss9_GH1 growth hormone I gene, complete cds	1188	1188	99%
AY614007.1	Salmo salar isolate Ss8_GH1 growth hormone I gene, complete cds	1188	1188	99%
AY614006.1	Salmo salar isolate Ss7_GH1 growth hormone I gene, complete cds	1188	1188	99%
AY614005.1	Salmo salar isolate Ss6_GH1 growth hormone I gene, complete cds	1188	1188	99%
AY614004.1	Salmo salar isolate Ss5_GH1 growth hormone I gene, complete cds	1188	1188	99%
AY614010.1	Salmo salar isolate Ss11_GH1 growth hormone I gene, complete cds	1182	1182	99%
AY614009.1	Salmo salar isolate Ss10_GH1 growth hormone I gene, complete cds	1182	1182	99%
AY61	alar isolate Ss2_GH1 growth hormone I gene, complete cds	1182	1182	99%
AY614003.1	Salmo salar isolate Ss3_GH1 growth hormone I gene, complete cds	1181	1181	99%
X61938.1	Salmo salar gene for growth hormone I	1177	1177	99%
JN241634.1	Salmo trutta caspius growth hormone gene, complete cds	137	137	9%

Show report for AY614010.1

Fig. 8: Sequence analysis of the Growth hormone gene in *Salmo trutta* growth hormone 1 gene, intron C, with sequence's other salmonids in Gene Bank. Scores denote conserved nucleotides.(100%) and *Salmo trutta caspius* (9%)

GH1, also in the *Salmo salar* there are five introns and six exons, but the length of introns in *Salmo salar* had been different with *salmo trutta caspius* GH gene. The analysis and sequencing provided valuable information about the mode of evolution of these DNA sequences. We tested segregation of the variants of GH genes in salmonids, and demonstrated that they are alleles at a common ancient (Fig. 6).

Population studies using the GH1 alleles, showed highly significant frequency between population studies using the GH1 alleles *Salmo trutta caspius*, and *Salmo salar* respectively. Interspecies sequence divergences of this GH-1 intron are higher than for GH-1 exons. Evolutionary divergences among alleles and among species for GH-1 genes measured by aligning salmonids, including *Salmo trutta caspius* and other salmonids sequences.

Role of GH gene relationship in salmo trutta caspius and salmo trutta: The brown trout (*Salmo trutta* L.) show variation in its tendency to migrate (Hindar *et al*.,1991). Sea trout (*Salmo trutta*) are anadromous. That is, it migrates to the salt water environment to feed and returns to streaming freshwater to span (L'Abee - Lund *et al*., 1989).The *Salmo trutta* is caught mainly coastal home waters, although some individuals may have travelled in the open sea 100-600 km away from their Home river (Skrochowski, 1969; Toivonen *et al*., 1978). Together mature and immature *Salmo trutta* tend to migrate annually between sea and river (Sturlaugsson and Johannsson, 1996). *Salmo trutta* leave the river for the first time at 2-7-year-old smolts (Jonsson, 1982). In related to, Berg (1962), had reported that *Salmo trutta caspius* probability, 12000 to be 14000 years old ago, has been travelled from White sea in Russia to the Caspian sea, they selected south and south west of the Caspian sea that was related to deep. Moreover, Berg (1962), Reported that some truth is common ancient with *Salmo salar*, Salmo trutta Caspius, *Salmo trutta fario* and *onchorhyncus mykiss*, in fact, *Salmo trutta caspius* they originated from *Salmo trutta* and *Salmo salar* . However, with sequence of growth hormone gene that it is a marker polymorphism. We could find a relationship between *Salmo trutta cassius* with *Salmo salar* and *Salmo trutta*, respectively. In related to, the sequence of GH gene in *Salmo trutta caspius* was aligned with the Gene Bank program with other salmonids, that including 23% homology with *Salmo trutta* intron d length of the 1096 bp. (Fig. 7) and 9% *Salmo trutta* intron C, length of the 810 bp. (Fig. 8). Furthermore, *Salmo trutta caspius* has been high homology with *Salmo salar* (100%). However; there was the not full length of GH gene in *Salmo trutta* for getting exactly result about between *Salmo trutta* and *Salmo trutta caspius.*

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