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Comparison of the Variation Intron Sequences of Growth Hormone Gene in *Salmo trutta caspius, Salmo salar* and *Salmo trutta*

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

Abstract: The *Salmo trutta caspius* are used for studies of the Growth Hormone (GH) gene, this gene have two major functions, first, The studies for genetics polymorphism in *Salmo trutta caspius* with other salmonids, second, the GH gene cause of increasing metabolism and growth body system in fish, especially salmonids. In this research, we found the full length of the growth hormone gene of *Salmo trutta caspius*. In related to, first, we isolated DNA genomics from fin tissue for studies about PCR techniques, and then were designed pair of primers from some salmons of GH genes accessed in the Gene Bank. These primers amplified almost, fragments of 810, 310 and 920 bp. The fragments after purification on the gel electrophoresis were sent to the sequencing. In this study we had aim that amount of similarity between *Salmo trutta caspius*, *Salmo salar* and *Salmo trutta*. However the results of morphology are shown that these species had strong similarity with them, but they have lived in the different region. *Salmo trutta caspius* lives in the Caspian Sea in the north of Iran, but for usually *Salmo salar* and *Salmo trutta*, live in the Atlantic Ocean and White Sea. The fragment sequences were compared between *Salmo trutta caspius*, *Salmo salar* and *Salmo trutta* by the GH gene. The results are shown that there is a high homology between *Salmo salar* and *Salmo trutta* regarding GH gene.

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

INTRODUCTION

The family Salmonidae comprises eleven genera and includes salmon, trout, charr, freshwater whitefishes, ciscos and graylings (Nelson, 2006). The Salmons are important for aquaculture and considerable of economics. Furthermore, Salmonids are most of the main food in Asia, especially Iran, in Europe contain most of the countries and America and Canada (Davidson *et al*., 2010), *Salmo trutta caspius* species are living in the Caspian Sea. This species is very rarely in the world. Because *Salmo trutta caspius* is very sensitive to pollution in the water. The adult female salmons also for spawning should be travel to fresh water. So, is very existence study and researches on the *Salmo trutta caspius*. In related to we examined regard polymorphism of the *Salmo trutta caspius* and other Salmonids for getting result amount of the relationships between *Salmo trutta caspius* and other Salmons. In the Europe, the Middle East, Western Asia, and parts of North Africa. From North to South, its range extends from northern Norway and north-eastern Russia, to the Atlas Mountains of North Africa. Other trout species have been also investigated. Some major scientific questions can be explored using Salmonid genomes. The lot of genes can be investigated to Salmons such as mitochondrial DNA (Karlsson *et al*., 2009), microsatellites (KEVIN *et al*., 2002) and growth hormone genes (Gross and Nilsson, 1995; Gross *et al*., 1996; Ryynanen and Primmer, 2004; Agellon *et al*., 1988). In related to, we examined Growth Hormone (GH) gene for detection polymorphism and common ancestor in Salmons. The GH gene of the *Salmo salar* and *Salmo trutta* was selected to be the reference sequence for all Salmonids on the basis of its importance for the aquaculture industry and because so much previous work has been carried out at the GH genes on this species.

The GH gene in Solminds are two types, type I and II, resulting from their polyploid ancestry (Johansen *et al*., 1989; Forbes *et al*., 1994) found sequence of the full length of GH gene in *Salmo trutta caspius* (Accession number, JN241634.1) (Rezaei *et al*., 2011a, b). We aligned GH gene in the *Salmo trutta caspius* with *Salmo salar* and *Salmo trutta*, by the NCBI Network system program. Results are shown there is a high homology with *Salmo salar* (97%), there is also a high homology with *Salmo trutta* intron C, intron D, GH1 and intron D GH2. However we cannot get exactly result from *Salmo trutta*, because there was not full length of GH gene of *Salmo*

Corresponding Author: Abolhasan Rezaei, Islamic Azad University Tonekabon Branch-Iran, P.O. Box 4864161187, Tonekabon, Iran

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S.salar                  GTAAGCCTGCTTTTTCTGTCTATTTCTTTTTTCAGTGGGA   740
S.trutta caspius(intron1)GTAAGCCTGCTTTTTCTGTCTATTTCTTTTTTtAGTGGGA   40
S.trutta (intron d)      ....................................       0
S.trutta (intron C)      ....................................       0
Consensus
S.salar                  AGTCAGTGTACCATTTAGTACAATTTAACTTACACATTTA   780
S.trutta caspius(intron1)AGTCAGTGTACCATTTAGTACAgTTTAACTTACACATTTA   80
S.trutta (intron d)      ....................................       0
S.trutta (intron C)      ....................................       0
Consensus
S.salar                  ATCACTGAGGCAGGGGCCAACACGGCAGAGAAAAGTGAAC   820
S.trutta caspius(intron1)ATCACTGAGGCAGGGGCCAACACGGCAGAGAAAAGTGAAC   120
S.trutta (intron d)      ....................................       0
S.trutta (intron C)      ....................................       0
Consensus
S.salar                  AAGTATTCTACTACTATGAGGTTATAAATCTATTGACACA    860
S.trutta caspius(intron1)AAGTATTCTACTACTATGAGGTTAT.AATCTATTGACACA    159
S.trutta (intron d)      ....................................        0
S.trutta (intron C)      ....................................        0
Consensus
S.salar                  GAACCACCTGCTTTAACAACCTAACTATGTGATCTATAAC    900
S.trutta caspius(intron1)GAACCACCTGCTTTAACAAC CTAACTATGTGATCTATAAC   199
S.trutta (intron d)      ....................................        0
S.trutta (intron C)      ....................................        0
Consensus
S.salar                  ATTTACATTTGAGTCGTTTAGCAGACGCTCTTATCCAGAG    940
S.trutta caspius(intron1)ATTTACATTTGAGTCaTTTAGCAGACaCTCTTATCCAaAG    239
S.trutta (intron d)      ....................................        0
S.trutta (intron C)      ....................................        0
Consensus
S.salar                  CGACTTACAGGAGCAATTAGGGTTAAGTGCCTTGCTCAAG    980
S.trutta caspius(intron1)CGACTTACAGGAGCcATTAGGGTTAAGTGCCTTGCTCAAG    279
S.trutta (intron d)      ....................................        0
S.trutta (intron C)      ....................................        0
Consensus
S.salar                  GGCACGTCGACAGATTTCTCACCTAGTCAGCTCAGGGATT    1020
S.trutta caspius(intron1)GGCACaTCGACAGATTTCTCACCTAGTCAGCTCAGGGATT    319
S.trutta (intron d)      ....................................        0
S.trutta (intron C)      ....................................        0
Consensus
S.salar                  GAAACCAGTAACCTTTCAATTACTTACCCAACGCTCTTAA   1060
S.trutta caspius(intron1)GAAACCgGTAACCTTTCAATTACTTACCCAACGCTCTTAA   359
S.trutta (intron d)      ....................................       0
S.trutta (intron C)      ....................................       0
S.salar                  CCGCTAGGCTATTGGTGTTCGATGGCTGAGAATATCTAAC   1100
S.trutta caspius(intron1)CCGCTgGGCTATTGGTGTaCaATGGCTGAGAATATCTAAC   399
S.trutta (intron d)      ....................................       0
S.trutta (intron C)      ....................................       0
Consensus
S.salar                  TAATGTATCTCACCATAATTCGACTTACTCGTTTTATACA   1140
S.trutta caspius(intron1)TAATGTATCTCACCATAATTCGACTTACTCGTTTTATACA   439
S.trutta (intron d)      ....................................       0
S.trutta (intron C)      ....................................       0
Consensus
S.salar                  TTTGTTATTTTC.....TCTTTCTTTTAG              1164
S.trutta caspius(intron1)TTTcTTATTTTatttaaTCTcTCTTTTAG              468
S.trutta (intron d)      ...........................                0
S.trutta (intron C)      ...........................                0
Consensus
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Fig. 1: The sequence first intron identity in the GH gene between *Salmo trutta caspius*, *Salmo salar*, *Salmo trutta* intron D and *Salmo trutta* intron C, was compared by the DNAMAN genetics program. The results are shown that amount of similarity *Salmo trutta caspius* with *Salmo salar* is high but there weren't any similarity with *Salmo trutta* introns.The underlined sequences are mini and microsatellites in the fragments of *Salmo trutta caspius*, *Salmo salar* GH gene

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S.salar                   CTTTGTAAGACAGCTTTTGAATCTTCTTTTGACATATCAA   1340
S.trutta caspius(intron2)....GTAAGACAGCTTTTGAATCTTCTTTTGACATATCAA   36
S.trutta (intron d)       ....................................      0
S.trutta (intron C)       ....................................      0
Consensus
S.salar                   ATAGTGTATCAATGA.TGTTCTTCTTCTTGTAGACAGTGT   1379
S.trutta caspius(intron2)ATAaTGTATtAATGAtTGTTCTTCTTCTTGTAGACAGTaT   76
S.trutta (intron d)       ....................................      0
S.trutta (intron C)       ....................................      0
Consensus
S.salar                   CCTCTTTACACAA.CCCTCGTGGCAACAACAAAAAAATCT   1418
S.trutta caspius(intron2)CCTCTTTACACAAcCCCTCGcGGCtAaAAaAAAAcAAcag   116
S.trutta (intron d)       ....................................      0
S.trutta (intron C)       ....................................      0
S.salar                   CTCTCCCTTCTTTGTGATTT   1438
S.trutta caspius(intron2)aaaatCtcTCTc........    128
S.trutta (intron d)       ...................       0
S.trutta (intron C)       ...................       0
Consensus
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Fig. 2: The sequence second intron identity in the GH gene between *Salmo trutta caspius*, *Salmo salar*, *Salmo trutta* intron d and *Salmo trutta* intron C, was compared by the DNAMAN program. The results are shown that amount of similarity *Salmo trutta caspius* with *Salmo salar* is high but there weren't any similarity with *Salmo trutta* introns. The underlined sequences are mini and microsatellites in the fragments of *Salmo trutta caspius, Salmo salar* GH gene

trutta in the Gene Bank. The objectives of this research, studies of polymorphism between *Salmo trutta caspius* populations and amount of relationships with *Salmo salar* and *Salmo trutta*, moreover, the capability transforming GH gene in Salmons for our future planning.

MATERIALS AND METHODS

The *Salmo trutta caspius* used the experiment originated from two rivers in Iran (the river of Dohezar and Sardabroud in the North of Iran). These fishes had been 3 years age old . They were anesthetized with MS2220 and extracted of blood from 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 MS2220 (1:10,000) as an anesthetic to minimize stress.

Process of designing primers: For designing primers we had to use some sequences from Gene Bank, NCBI Network system. In related to, we thought the sequences of *Salmo salar* GH gene have been high homology with *Salmo trutta caspius*, because *Salmo salar* and also other bony fishes have same shape and morphology with *salmo trutta caspius*, therefore we had risk and was synthesized primers. Also, these sequences were around 4700 nucleotides, so, for getting high performance, we designed primers some part of the full length of GH gene of *Salmo salar* by the NCBI Network program and DNAMAN genetics program. These primers amplified 330, 850, 910 bp. from first to end of the GH gene of *Salmo trutta caspius*.

The PCR programs: The PCR reaction used 10 microgram 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 (2.5 mM each), 5 μL 10X Chrom Taq Assay buffer, 0.5 μL ChromTaq enzyme (3 U/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. Two to ten μL of each PCR reaction were run on 1% agarose gels in TAE buffer containing ethidium bromide. One μL 500 bp, DNA ladder (Gibco-BRL) was used as a size standard. Then the PCR products after purification by the Chromous kit purification were sent to the Choromous Geni Company-India for doing sequence.

RESULTS

Analysis of the polygenetics of GH gene in the *salmo trutta caspius*: The primers were designed to four segments of the GH gene, because full length of the GH gene in the Salmo trutta caspius has been around 4700 nucleotides. The fragments contain of the introns. In related to, we compared between of the *Salmo trutta caspius* GH gene with 33 sequences reporting to the NCBI Network system by DNAMAN gene program. The introns which ranged from 1 to 2541 nucleotides. According to for introns contains: (45.. 512), (653.. 803), (921.. 1063), (1220.. 1473), (1621.. 1844).

Comparison of the GH introns in the *salmo trutta caspius* with other salmons: In related to, compared between GH introns in the *Salmo trutta caspius* and other Salmons, including *Salmo salar* population by the DNA gene program. Therefore the results are shown; there is low homology between in the introns. In the Fig. 1, first intron aligned with *Salmo trutta* and *Salmo salar* populations. We observed that there is high homology between *Salmo salar*. Regarding to the *Salmo trutta* homology was not significant, in the Fig. 2, second intron compared with Salmons population, which high

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S.salar          CAAAGGTTTTTAACTCAATC                  2294
S.trutta caspius(intron3) ........TTTggCgggtTg    12
S.trutta (intron C)       CAAAGGTTTTTAACTCAATC    730
S.trutta (intron d)       ...................     0
Consensus
S.salar                   ATGTAAATAGGGAATCTCAAGCTGTACAATACAACGCAAC   2334
S.trutta caspius(intron3) gTGTAAATAGGGAATCTCAAGCTGTACAATACAACGCAAC    52
S.trutta (intron C)       ATGTAAATAGGGcATCTCAAGCTGTACAATACAACGCAAC    770
S.trutta (intron d)       ....................................    0
Consensus
S.salar                   TTCATTTTCCAATAATCTGTGGTTTCTCTACATCTACACA   2374
S.trutta caspius(intron3) TTCATTTTCCAATAATCTGTGGTTTCTCTACAcacACAg.   92
S.trutta (intron C)       TTCATTTTCCAATAATCTGTGGTTTCTCTACAcacACAag   810
S.trutta (intron d)        ....................................  0
Consensus
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Fig. 3: The sequence third intron identity in the GH gene between Salmo trutta caspius, *Salmo salar*, *Salmo trutta* intron D and *Salmo trutta* intron C, was compared by the DNAMAN program. The results are shown that amount of similarity *Salmo trutta caspius* with *Salmo salar* is high. There is also similarity with *Salmo trutta* intron C. The underlined sequences are mini and microsatellites in the fragments of *Salmo trutta caspius, Salmo salar* and *Salmo trutta* GH gene

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S.salar          CAAGGTAAAGAAAGGAGGGAGAACAATGACCATTTGTGGT                2574
S.trutta caspius(intron4) .....TAAAGAAAGGAGGGAGAACAATGACCATTTGTGGT     35
S.trutta (intron d)       ....GTAAAGAAAGGAGGGAGAACAATGACCATTTGTGGT     36
S.trutta (intron C)       ....................................     810
Consensus
S.salar                   GCCACACTTTGTGCACTGTAAACCCCAAGGCATTTTTAAC     2614
S.trutta caspius(intron4) GCCACACTTTGTGCACTGTAAACCCCAAGGCATTTTTAAC     75
S.trutta (intron d)       GCCACACTTTGTGCACTGTAAACCCCAAGGCATTTTTAAC     76
S.trutta (intron C)       ....................................     810
Consensus
S.salar                   TCAAATACTTCTAGTAAGTTGAACTCAAAGTCAATGAAAA     2654
S.trutta caspius(intron4) TCAAATACTTCTAGTAAGTTGAAgTtgtg...........      104
S.trutta (intron d)       TCAAATACTTCTAGTAAGTTGAACTCAAAGTCAATGAAAA     116
S.trutta (intron C)       ....................................      810
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Fig. 4: The sequence fourth intron identity in the GH gene between *Salmo trutta caspius*, *Salmo salar*, *Salmo trutta* intron D and *Salmo trutta* intron C, was compared by the DNAMAN program. The results are shown that amount of similarity *Salmo trutta caspius* with *Salmo salar* is high. There is also similarity with *Salmo trutta* intron D. The underlined sequences are mini and microsatellites in the fragments of *Salmo trutta caspius, Salmo salar* and *Salmo trutta* GH gene

homology with *Salmo salar* sequences but low homology with sequences of the *Salmo trutta*. Also we compared third and fourth fragments intron for finding amount of homology between Salmons , that which high homology regarding *Salmo salar* and *Salmo trutta* populations (Fig. 3 and 4). Fifth intron also aligned but only there were similarity with *Salmo salar*. Regarding to *Salmo trutta* was not observed any homology with *Salmo trutta caspius* GH gene (Fig. 5).

DISCUSSION

Annotations introns fragment of GH gene in the salmo trutta caspius: The GH gene in the *Salmo trutta caspius* has 5 introns in the full length. While in the *Salmo salar*, GH gene also have 5 introns, but the length of introns in the *Salmo trutta caspius* are shorter than *Salmo salar* GH gene. The introns had also been strong homology with GH gene in the bony fishes, specially, *Salmo salar*, *Onchorhynchus mykiss* and *Salmo trutta*. However the information regarding polymorphism of the GH gene in fish is rather scarce (Park *et al*., 1995). Identified polymorphism in intron D of the GH 1 gene in the *Salmo trutta*, *Salmo trutta* intron C (Phillips *et al*., 2004), *Chinook salmon*, polymorphism was found also in *Pink salmon* GH2 intron C (Spruell *et al*., 1999), intron C of the *Coho salmon* GH1 gene that is due to a variable number of copies of a 31 nucleotides repeat. (Forbes *et al*., 1994). Nucleotide and repeat polymorphism in GH introns C and D of *Oncorhynchus masou* was described by McKay *et al*. (1998). Differences in the number of repeat monomers also resulted in polymorphism in the third intron of the barramundi GH gene (Yowe and Epping, 1996). Sequence analysis of GH genes from different groups of teleosts have both fundamental and practical significance. It may result in better understanding of GH gene evolution in teleosts and allow for tracing the origin of the fifth intron. Identifying possible allelic variations may also prove useful as

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Consensus
S.salar                  GACATGCACAAGGTGCAAAACCATGTTGCCTTCTATTTCA   3829
S.trutta caspius(intron5).....GTGCAAAACCATGTTGCCTTCaATTTCA   28
S.trutta (intron d)      ....................................   1096
S.trutta (intron C)      ....................................   810
Consensus
S.salar                  TGTGCCTTCCTATATTTTCTACAGTGCG....TTTCTTGT   3865
S.trutta caspius(intron5)TGTaCCTTCCTATATTTTtTACAGTGCGttgtTTTtTTGT   68
S.trutta (intron d)      ....................................   1096
S.trutta (intron C)      ....................................   810
Consensus
S.salar                  GCTCTCTATTGCAAAGTATCTTTGGGTCTTTAACCCATAT   3905
S.trutta caspius(intron5)GCTCTCTATTGCAAAGTATCTTTGGGTCTTTAACCCATAT   108
S.trutta (intron d)      ....................................   1096
S.trutta (intron C)      ....................................   810
Consensus
S.salar                  ATTATTACTATTATTGTTCATTGATCAAGACTGTTCTCGA   3945
S.trutta caspius(intron5)ATTATTACTATTATTGTTCATTGATCAAGACTGTTCTCGA   148
S.trutta (intron d)      ....................................   1096
S.trutta (intron C)      ....................................   810
Consensus
S.salar                  GAAAGGTCTAGTGACCTAGAACACTCACATTAAAATGTGT   3985
S.trutta caspius(intron5)GAAAGGTCTAGTGACCTAGAACACTCACATTAAAATGTGT   188
S.trutta (intron d)      ....................................   1096
S.trutta (intron C)      ....................................   810
Consensus
S.salar                  CAACTATAACCCATTCTTCTATTTTTCCCCCAAGGTCGAG   4025
S.trutta caspius(intron5)CAACTATAACCCATTCTTCTATTTTTtCCCCcaag....  224
S.trutta (intron d)      ..... 1096
S.trutta (intron C)      ..... 810
Consensus
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Fig. 5: The sequence fifth intron identity in the GH gene between *Salmo trutta caspius*, *Salmo salar*, *Salmo trutta* intron D and *Salmo trutta* intron C, was compared by the DNAMAN genetics program. The results are shown that amount of similarity *Salmo trutta caspius* with *Salmo salar* is high but there weren't any similarity with *Salmo trutta* introns. The underlined sequences are mini and microsatellites in the fragments of *Salmo trutta caspius, Salmo salar* GH gene

genetic markers for commercially important traits, similar to recent findings in domesticated animals.

First intron: The first intron sequence ranged from nucleotide of 45 to 512, and alignment was 377 bp. The analysis of first GH intron between *Salmo trutta caspius* and *Salmo salar*, has been strong homology but there is not any homology with *Salmo trutta*. These results are shown that the full length of intron D in the *Salmo trutta* was not district the GH gene in the *Salmo trutta caspius* and also *Salmo salar*. Many mini- and microsatellite repeat sequences were found throughout the noncoding region of the length of first intron which results in extensive allelism due to different numbers or length of repeats. That which includes, repeats (CTTTT..). Interestingly , the three nucleotide and tetra nucleotide tandem repeat (ATT and ATTT)n found in the first intron of *Salmo salar* GH gene is similar to the three and tetramer tandem repeat (ATT) in the first intron of *Salmo trutta caspius* GH gene.

Second intron: In the second intron sequences is that it contains in its sequence the three and tetranucleotides repeat, CTT and CTTT which are in the length of second intron in *Salmo salar* and also *Salmo trutta* caspius. Another interesting feature of this region of rich A nucleotides in the end of length *Salmo trutta caspius* while in *Salmo salar* region of rich CT is high. On the other hand, the length of second intron GH in *Salmo trutta caspius* is 128 nucleotides while in the *Salmo salar* is 136 nuucleotides. However, there is not any homology between *Salmo trutta caspius* second intron with *Salmo trutta* intron D and C. The length of the sequences aligned by DNAMAN program genetics, results are shown that there is minor variation between *Salmo trutta caspius* and *Salmo salar*, but the length of the *Salmo salar* is more than *Salmo trutta caspius* length of second intron GH.

Third, fourth and fifth fragments intron: The extensive polymorphism in the third, fourth and fifth fragment, were found a lot mini and microsatellites in the different regions of the fragments which has potential to serve as genetic markers in genetic selection for desirable traits in the Salmons GH gene. In the third intron of *Salmo trutta caspius* ranged of 92 nucleotides but in the *Salmo salar* and *Salmo trutta* intron C were 100 nucleotides, regarding *Salmo trutta* intron D there weren't any nucleotides in span of that nucleotides. The sequences also were aligned by DNAMAN genetics program there are high homology between sequences, however length of the *Salmo trutta caspius* was shorter than other two sequences. In the other feature, there are region of the rich AT and TC in the length of sequences. The fourth intron GH aligned also by the DNAMAN program, results are shown that strong

homology between *Salmo trutta caspius* and *Salmo salar* however is shorter than *Salmo trutta caspius* regarding fourth intron GH. The results also are shown that there are mini and microsatellites, specially nucleotides rich of AAT and TAA in the fourth and fifth intron. In related to, intron C had high homology with *Salmo trutta caspius* and *Salmo salar*, but the length of the fourth intron in *Salmo trutta caspius* was shorter than intron C and *Salmo salar* length. Regarding to the fifth intron there were also strong homology with *Salmo salar* but there weren't any homology with *Salmo trutta* intron D and C.

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