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## Phylogenetic analysis of NADH dehydrogenase subunit 1(NADH 1) gene in *Salmo trutta caspius*

**Abolhasan Rezaei**

### Abstract

The NADH 1 gene in the *Salmo trutta caspius* has been sequenced, characterized and deposited in Genbank, Accession Number LC011387. In this study we compared NADH 1 gene between *Salmo trutta caspius*, *Salmo trutta trutta*, accession number (AM910409.1), *Salmo salar* (KF792729.1) and *Oncorhynchus mykiss* (DQ288268.1) by DNAMAN program and NCBI Network system separately. According to DNAMAN program analysis, we found 23 SNPs and 98% similarity between *Salmo trutta caspius* and *Salmo trutta trutta*, 67 SNPs and 92% similarity between *Salmo trutta caspius* and *Salmo salar*, 126 SNPs and 86% similarity between *Salmo trutta caspius* and *Oncorhynchus mykiss*. Amino acid sequences also were determined by DNAMAN program. Between *Salmo trutta caspius* and *Salmo trutta trutta*, 3 SNPs, between *Salmo trutta caspius* and *Salmo salar* 14 SNPs, and between *Salmo trutta caspius* and *Oncorhynchus mykiss*, 17 SNPs were observed. In this study we concluded that the rate of homology between *Salmo trutta trutta* and *Salmo trutta caspius* is more than its homology with *Salmo salar* and *Oncorhynchus mykiss*, so these results show that *Salmo trutta caspius* had originated from *Salmo trutta*.

**Keywords:** *Salmo trutta caspius*, *Salmo salar*, *Salmo trutta*, *Oncorhynchus mykiss*, NADH 1 gene

### 1. Introduction

NADH dehydrogenase (ubiquinone) is an enzyme of the respiratory chains in myriad of organisms from bacteria to humans. It catalyzes the transfer of electrons from NADH to its coenzyme and, in eukaryotes, it is located in the inner mitochondrial membrane. NADH 1 gene was investigated and characterized in Hucho populations [27]. They used two mitochondrial loci (control region and NADH 1) in 47 *Huchen* samples to study genetic diversity and phylogeographic structure of *Huchen* populations. Neave, 1958 [12] proposed that the common ancestor of rainbow trout was the first to diverge from the *protoOncorhynchus* evolutionary line, which then radiated to form the seven extant Pacific salmon species. They found a relationship between brown trout and *Oncorhynchus mykiss* when compared different subunits of NADH genes and growth hormone genes [14], using polymerase chain reaction, reported that two mtDNA loci, cytochrome and NADH 1, were amplified in 14 individuals. A similar comparison with the NADH 1 sequences revealed a similar ratio in rainbow trout, chum, sockeye and *Salmo salar*. Generally, NADH in mitochondria of *Salmons*, especially *Salmo trutta caspius* has seven subunits (NADH 1, NADH 2, NADH 3, NADH 4, NADH L, NADH 5 and NADH 6), which have different size in full length. The full length of NADH 1 gene in *Salmo trutta*, *Salmo salar* and *Oncorhynchus mykiss* was amplified, sequenced and deposited in GenBank (NCBI-Network system). According to their sequences, NADH 1, have been approximately 970-1000 bp in full length. To compare the phylogeny in brown trout, three methods were used to compare NADH 1 gene in *Salmo salar* and *Oncorhynchus mykiss* (parsimony, maximum likelihood, and neighbour-joining distance analyses) which found a close relationship between them. mtDNA NADH 3 gene region is useful as a genetic marker for stock identification and phylogeographic study because NADH 3 gene is conserved across all organisms and has a fast rate of sequence evolution [10, 28]. *Salmo trutta caspius* was originated from south of Caspian Sea, Iran. They are on the IUCN Red List of Threatened Species. Therefore, studies on the *Salmo trutta caspius* are very important. The aim of this research was to study the phylogenetic of *Salmo trutta caspius* by comparing its NADH 1 gene with other *Salmonids*. NADH 1 gene was suitable for finding the relationships within *Salmo trutta caspius* species and between *Salmo trutta caspius* and other species of *Salmonids* such as *Salmo trutta*, *Salmo salar* and *Oncorhynchus mykiss*.

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## 2. Materials and Methods

### 2.1 Fish samples and DNA extraction

In June of 2014, blood samples were excised from *Salmo trutta caspius* collected from the rivers of Tonekabon, Iran, for a total of 30 fish including male and female samples that were three years old. Samples were stored at -80°C, or kept in 80% ethanol at room temperature until DNA extraction. DNA was extracted from approximately 70 mg of the stored specimens with a QIAGEN Blood and Cell Culture DNA Midi Kit (Qiagen, Germany) according to the manufacturer's instruction. A UV spectrophotometer (Eppendorf, Germany) was used to determine the quantity and quality of the isolated DNA. DNA concentration was estimated by measuring the absorbance at 260 nm wave length. Protein contamination was estimated by the ratio of absorbance at 260 nm and 280 nm wave lengths [20].

### 2.2 PCR amplification

NADH 1 gene was amplified with polymerase chain reaction (PCR) technique in 50 µl reaction mixture containing 1-2 ng DNA, PCR Buffer 1 X, 1 unit Taq DNA polymerase (Roche, Germany), 0.2 mM dNTPs and 10 pMol forward and Reverse primers. The condition of PCR amplification using a thermo cycler instrument (BioRad, USA) was as follow: denaturation at 94°C for 5 min, following by 35 cycles of 59°C for 1 min, and 72°C for 5 min, with final-extension at 72°C for 7 min. The amplified DNA fragments were determined for the size and quality with 1.5% agarose-gel electrophoresis and ethidium bromide staining, then purified by the QIAquick PCR Purification Kit (Qiagen, Germany).

### 2.3 Sequencing and phylogenetic analysis

The amplified DNA fragments sequenced which carried out by ABI PRISM dye terminator cycle sequencing kit (PE Biosystem, USA) according to the manufacturer's instruction. After sequencing, the data were deposited in GenBank, accession number LC011387.

Bioinformatics analysis:

The sequence of NADH 1 gene was analysed by DNAMAN (Version 8, Demo) program and NCBI Network system and compared between *Salmo trutta caspius* and other *Salmonids*, based on the sequences that were reported in GenBank, to find the percent of homology between *Salmonid* species.

## 3. Results

### 3.1 Sequencing of NADH 1 gene in *Salmo trutta caspius*

The full length of NADH 1 gene was sequenced and deposited in GenBank, accession number LC011387. NADH 1 gene in *Salmo trutta caspius* is 969 bp in length (Figure 1).

### 3.2 Phylogenetic analysis of NADH 1 gene between *Salmo trutta caspius*, *Salmo trutta trutta*, *Salmo salar* and *Oncorhynchus mykiss*

In this research we used three species of *Salmonids* for phylogenetic analysis between them. These sequences of

NADH 1 gene had reported from *Salmo trutta trutta* (accession number AM910409.1), *Salmo salar* (KF792729.1) and *Oncorhynchus mykiss* (DQ288268.1) in GenBank.

```
1.ATGATTACCACCTAATTACCCACGTTGTTAACC
CACTCGCATAACATCATCCCCATCCTTTTAGCAGTT
GCTTTTCCTCACCCCTACTCGAACGAAAAGTCCTTGG
GTATATACAACCTTCGAAAAGGACCAAACATCGTCG
GTCCATACGGATTACTTCAACCTATCGCGGACGGC
CTAAAACATTTTATTAAGAACCAGTCCGACCATC
CACCTCCTCCCCCTTCTATTTCTCGCTACACCTA
TACTTGCCCTCACACTCGCACTAACCCCTATGAGCC
CCTATACCCATCCCCTACCCCATACGGACCTAAA
TCTAGGGGTACTATTTATCCTCGCACTCTCCAGCC
TGGCCGTGTATTCTATTTTAGGCTCAGGATGAGCT
TCAAATTCCAAATACGCTCTAATTGGAGCTCTACG
AGCAGTAGCACAACCATCTCCTACGAAGTTAGCC
TAGGACTAATCTTACTCGGCGTAATTATCTTTACA
GGAGGATTTACACTCCAAACCTTTAACGTAGCCCA
AGAAAGCATCTGACTACTCGTACCAGCCTGACCCC
TTGCCGCCATATGGTATATTTCTACTCTAGCTGAA
ACAAACCGCGCACCCCTTTGACCTCACAGAAGGAGA
ATCAGAGTTAGTCTCGGGTTAATGTAGAATACG
CCGAGGGCCATTTCGCCCTTCTTTCTAGCCGAA
TACGCTAATATCCTTCTAATAAATACTCTCGAC
CATTTTATTTCTTAGGCGCATCCCACATCCCCGCC
TCCCCGAATTAACAGCCGTAATCTAATAACAAAA
GCCGCCCTCCTTTCCGTTGTATTTTATGAGTACG
AGCCTCCTACCCCGATTTTCGATACGACCAGCTCA
TACTTGTGTTGAAAAAATTCTTACCTTTAACA
CTAGCTCTTGTCTGTGGCACCTAGCGCTTCCAAC
CGCAACAGCAGGCCTCCCCCCTCA. 969
```

**Fig 1:** The full length of NADH 1 gene in *Salmo trutta caspius* were deposited in GenBank, accession number, LC011387 with 969 bp.

We compared the NADH 1 gene between *Salmo trutta caspius* and *Salmo trutta trutta* (accession number AM910409.1), *Salmo salar* (KF792729.1) and *Oncorhynchus mykiss* (DQ288268.1) by DNAMAN program and NCBI Network system separately (Figures 2-4). According to DNAMAN program and NCBI Network system analysis, we found 23 SNPs and 98% similarity between *Salmo trutta caspius* and *Salmo trutta trutta*, 67 SNPs and 92% similarity between *Salmo trutta caspius* and *Salmo salar*, and 126 SNPs and 86% similarity between *Salmo trutta caspius* and *Oncorhynchus mykiss*. Amino acid sequences also were determined by DNAMAN program, and our data shows that between *Salmo trutta caspius* and *Salmo trutta trutta* 3 SNPs, between *Salmo trutta caspius* and *Salmo salar* 14 SNPs, and between *Salmo trutta caspius* and *Oncorhynchus mykiss*, 17 SNPs are identified (Figures 5-7).

s.t.caspius	ATGATTACCACCCTAATTACCCACGTTGTTAACCCACTCGCATACATCATCCCCATCCTTTTAGC	65
s.t.trutta	ATGATTACCACCCTAATTACCCACGTTGTTAACCCACTCGCATACATCATCCCCATCCTTTTAGC	65
Consensus	atgattaccaccctaattacccacgttgttaaccactcgcatacatcatccccatccttttagc	
s.t.caspius	AGTTGCTTTCCTCACCCTACTCGAACGAAAAGTCCTTGGGTATATACAACCTTCGAAAAGGACCAA	130
s.t.trutta	AGTTGCTTTCCTCACCCTACTCGAACGAAAAGTCCTTGGGTATATACAACCTTCGAAAAGGACCAA	130
Consensus	agttgcttttcctcacc tactcgaacgaaaagtccttgggtatataacaacttcgaaaaggaccaa	
s.t.caspius	ACATCGTCCGGTCCATACGGATTACTTCAACCTATCGCGGACGGCCTAAAACCTATTTATTAAGAA	195
s.t.trutta	ACATCGTCCGGTCCATACGGATTACTTCAACCTATCGCGGACGGCCTAAAACCTATTTATTAAGAA	195
Consensus	acatcgtccggtccatacggattacttcaacctatcgcgacggcctaaaactatttattaagaa	
s.t.caspius	CCAGTCCGACCATCCACCTCCTCCCCCTTCCTATTTCTCGCTACACCTATACTTGCCCTCACACT	260
s.t.trutta	CCGTTTCGACCATCCACCTCCTCCCCCTTCCTATTTCTCGCTACACCTATACTTGCCCTCACACT	260
Consensus	cc gt cgaccatccacctcctcccccttcctatttctcgcctacacctatacttgccctcacact	
s.t.caspius	CGCACTAACCCCTATGAGCCCTATACCCATCCCCTACCCTATTACCGACCTAAATCTAGGGGTAC	325
s.t.trutta	CGCACTAACCCCTATGAGCCCTATACCCATCCCCTACCCTATTACCGACCTAAATCTAGGGGTAC	325
Consensus	cgcactaacccctatg agccctatacccatccccctacc attac gacctaaatctaggggtac	
s.t.caspius	TATTTATCCTCGCACTCTCCAGCCTGGCCGTGATTCTATTTTAGGCTCAGGATGAGCTTCAAAT	390
s.t.trutta	TGTTTATCCTCGCACTCTCCAGCCTAGCCGTATATTCTATTTTAGGCTCAGGATGAGCTTCAAAT	390
Consensus	t tttatcctcgcactctccagcct gccgt tattctattttaggctcaggatgagcttcaaat	
s.t.caspius	TCCAAATACGCTCTAATTGGAGCTCTACGAGCAGTAGCACAAACCATCTCCTACGAAGTTAGCCT	455
s.t.trutta	TCCAAATACGCTCTAATTGGAGCTCTACGAGCAGTAGCACAAACCATCTCCTACGAAGTTAGCCT	455
Consensus	tccaaatacgctctaattggagctctacgagcagtagcacaaaccatctcctacgaagt agcct	
s.t.caspius	AGGACTAATCTTACTCGCGTAATTATCTTTACAGGAGGATTTACACTCCAAACCTTCAACGTAG	520
s.t.trutta	AGGACTAATCTTACTCGCGTAATTATCTTTACAGGAGGATTTACACTCCAAACCTTCAACGTAG	520
Consensus	aggactaatcttactc gcgtaattatctttacaggaggatttacactccaaacctt aacgtag	
s.t.caspius	CCCAAGAAAGCATCTGACTACTCGTACCAGCCTGACCCCTTGCCGCCATATGGTATATCTCTACT	585
s.t.trutta	CCCAAGAAAGCATCTGACTACTCGTACCAGCCTGACCCCTTGCCGCCATATGGTATATCTCTACT	585
Consensus	ccaagaaagcatctgactactcgtaccagcctgacccttgccgccatatggtatat tctac	
s.t.caspius	CTAGCTGAAACAAACCGCGCACCCCTTTGACCTCACAGAAGGAGAATCAGAAATTAGTCTCCGGGTT	650
s.t.trutta	CTAGCTGAAACAAACCGCGCACCCCTTTGACCTCACAGAAGGAGAATCAGAAATTAGTCTCCGGGTT	650
Consensus	ctagctgaaacaaaccgcgaccctttgacctcacagaaggagaatcaga ttagtctccggggtt	
s.t.caspius	TAATGTAGAATACGCCGAGGGCCATTGCGCCCTCTTCTTTCTAGCCGAATACGCTAATATCCTTC	715
s.t.trutta	TAATGTAGAATACGCCGAGGGCCATTGCGCCCTCTTCTTTCTAGCCGAATACGCTAATATCCTTC	715
Consensus	taatgtagaatacgccgagggccattcgccctcttctttctagccgaatacgctaataatccttc	
s.t.caspius	TAATAAATACACTCTCGACCATTTTATTTTTAGGGCGCATCCACATCCCCGCCCTCCCCGAATTA	780
s.t.trutta	TAATAAATACACTCTCGACCATTTTATTTTTAGGGCGCATCCACATCCCCGCCCTCCCCGAATTA	780
Consensus	taataaatacactctcgaccattttatt ttagggcgc tccacatccccgcc tccccgaatta	
s.t.caspius	ACAGCCGTAAATCTAATAACAAAAGCCGCCCTCCTTTCCGTTGTATTTTTATGAGTACGAGCCTC	845
s.t.trutta	ACAGCCGTAAATCTAATAACAAAAGCCGCCCTCCTTTCCGTTGTATTTTTATGAGTACGAGCCTC	845
Consensus	acagccgtaaatacctaataacaaaagccgccctcctttccgttgtatTTTTATGAGTACGAGcctc	
s.t.caspius	CTACCCCCGATTTGATACGACCAGCTCATACTTAGTTTGAAAAAAGCTTCTTACCTTTAACAC	910
s.t.trutta	CTACCCCCGATTTGATACGACCAGCTCATACTTAGTTTGAAAAAAGCTTCTTACCTTTAACAC	910
Consensus	ctacccccgattttgatacgaccagctcatacttagtttgaaaaa cttcttacctttaaacac	
s.t.caspius	TAGCTCTGTCTCTGTGGCACCTAGCGCTTCCAACCGCAACAGCAGGCCTCCCCCTCA.....	969
s.t.trutta	TAGCTCTGTCTCTGTGGCACCTAGCGCTTCCAACCGCAACAGCAGGCCTCCCCCTCAACTTTA	974
Consensus	tagctcttgtctct tg cacctagcgttccaaccgcaacagcaggcctcccc a	

s.t.caspius	Max score	Total score	Query cover	E value	Ident	Accession
s.t.trutta	1655	1655	100%	0.0	98%	55999

Fig 2: NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Salmo trutta trutta* (S.t. trutta) were analysed with DNAMAN program and NCBI Network system. There were 23 SNPs and 98% similarity between them.

s.t.caspius ATGATACCACCCTAATTACCCACGTTGTTAACCCACTCGCATACATCATCCCATTCTTTTAGC 65  
s.salar ATGACTACCACCCTAATTACCCACATTATTAACCCCTCGCATATATCGTCCCCGTTCTTTTAGC 65  
Consensus atga taccaccctaattaccac tt ttaacc ctgcata atc tccc t cttttagc

s.t.caspius AGTTGCTTTCTCACCCTACTCGAACGAAAAGTCCTTGGGTATATACAACCTCGAAAGGACCAA 130  
s.salar AGTCGCTTTCTCACCCTACTCGAACGAAAAGTCCTTGGATATATGCAACTTCGAAAGGGCCAA 130  
Consensus agt gctttcctcacc tactcgaacgaaaagtccttgg tatat caacttcg aaagg ccaa

s.t.caspius ACATCGTCGGTCCATACGGATTACTTCAACCTATCGCGGACGGCCTAAAACCTATTATTAAAGAA 195  
s.salar ACATCGTCGGTCCATACGGATTGCTTCAACCTATCGCGGACGGCCTAAAACCTATTATTAAAGAA 195  
Consensus acatcgtcgggtccatacggatt cttcaacctatcgcggacggcctaaaactatt attaaagaa

s.t.caspius CCAGTCCGACCAATCCACCTCCTCCCCCTTCTATTTCTCGCTACACCTATACTTGCCTTCAACT 260  
s.salar CCAGTTCGACCGTCCACCTCCTCCCCCTTCTATTTCTCGCTACACCTATACTTGCCTTACGCT 260  
Consensus ccagt cgacc tcacctcctcccccttctattttctcgtacacc atacttgcct ac ct

s.t.caspius CGCACTAACCTATGAGCCCCATATCCCATCCCCTACCCATTACGACCTAAATCTAGGGGTAC 325  
s.salar TCGCACTAACCTATGAGCCCCATGCCCATCCCCTACCCATTACGACCTAAATCTAGGGGTAC 325  
Consensus gcactaacct tgagccc at cccatccc taccattac gacctaaatct ggggtac

s.t.caspius TATTTATCCTCGCACTCTCCAGCCTGCGCGTGTATTCTATTTTAGGCTCAGGATGAGCTTCAAA 390  
s.salar TATTTATCCTCGCACTCTCCAGCCTAGCGCGTGTATTCTATTTTAGGCTCAGGATGAGCTTCAAA 390  
Consensus tattt tctcgcact tccagcct gccgtgtattctattttaggctcagg tgagcttcaaat

s.t.caspius TCCAAATACGCTCTAATTGGAGCTCTACGAGCAGTAGCACAAACCATCTCCTACGAAGTAGCCT 455  
s.salar TCTAAATATGCCCTAATTGGAGCTCTACGAGCAGTAGCACAAACCATCTCCTACGAAGTAGCCT 455  
Consensus tc aaata gc ctaattggagctctacgagcagt gcacaaacat tcttacgaagt agcct

s.t.caspius AGGACTAATCTTACTCGCGTAATTATCTTTACAGGAGGATTTACTCTCAAACCTTAAACGTAG 520  
s.salar TGGACTAATCTTACTCAGCGTAATTATCTTTACGGCGGATTTACTCTCAAACCTTAAATGTAG 520  
Consensus ggactaatcttactc gcgtaattatctttac gg ggatttacact caaacctt aa gtac

s.t.caspius CCCAAGAAAGCATCTGACTACTCGTACCAGCCTGACCCTTGCCGCCATATGGTATATTTCTACT 585  
s.salar CCCAAGAAAGCATCTGACTACTCGTACCAGCCTGACCCTTGCCGCCATATGGTATATCTCTACT 585  
Consensus cccaagaaagcatctgactactcgtaccagcctgacccttgccgccatattggtatat tctact

s.t.caspius CTAGCTGAAACAAACCGCGCACCTTTGACCTACAGAAGGAGAATCAGAGTTAGTCTCCGGGTT 650  
s.salar CTAGCTGAAACAAACCGTGCACCCTTTGACCTTACAGAAGGAGAATCAGAAATTAGTCTCCGGATT 650  
Consensus ctagctgaaacaaaccg gcaccctttgacct acagaaggagaatcaga ttagtctccgg tt

s.t.caspius TAATGTAGAATACGCCGAGGGCCATTTCGCCCTCTTCTTTCTAGCCGAATACGCTAATATCCTTC 715  
s.salar TAATGTAGAATACGCCGAGGGCCCTTCGCCCTCTTCTTTCTAGCCGAATACGCTAATATCCTTC 715  
Consensus taatgtagaatagccgagggcc ttcgccctcttctttctagccgaatagcctaataatccttc

s.t.caspius TAATAAATACACTCTCGACCATTATATTCTTAGGCGCATCCCACATCCCCGCCCTCCCCGAATTA 780  
s.salar TAATGAACACACTCTCGACCATTATATTCTTAGGCGCATCCCACATCCCCGCCCTCCCCGAATTA 780  
Consensus taat aa acactctcg ccatt tatt ttaggcgatcccacatccccgcc tccccgaatta

s.t.caspius ACAGCCGTAATCTAATAACAAAAGCCGCCCTCTTCCGGTGTATTTTTATGAGTACGAGCCTC 845  
s.salar ACAGCCGTAATCTAATAACAAAAGCCGCCCTCTTCCGGTGTATTTTTATGAGTACGAGCCTC 845  
Consensus acagccgtaaa ctaataacaaa gccgccctct tccggtgtatTTTTATGAGTACGAGCCTC

s.t.caspius CTACCCCGATTTCGATACGACCACTCATACTTAGTTTGAAAAAATTCTTACCTTTAACAC 910  
s.salar CTACCCACGATTTCGCTACGACCACTCATACTTAGTTTGAAAAAATTCTTACCTTTGACAC 910  
Consensus ctacc cgatttcg tacgacca ctataca ttagtttgaaaaa cttcttaccttt acac

s.t.caspius TAGCTCTGTCTCTGGCACCTAGCGCTTCCAACCGCAACAGCAGGCCTCCCCTCCCA..... 969  
s.salar TAGCCCTGTCTCTATGACACCTAGCACTTCCAACCGCAATGGCAGGCCTCCCCTCCCACTTTA 974  
Consensus tagc cttgtctct tg cacctagc cttccaaccgcaa gcaggctccc ccc a

s.t.caspius	Max score	Total score	Query cover	E value	Ident	Accession
s.salar	1373	1373	100%	0.0	92%	11609

Fig 3: NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Salmo salar* (S. salar) were analysed with DNAMAN program and NCBI Network system. There were 67 SNPs and 92% similarity between both sequences.

s.t.caspius ATGATTACCCACCCTAATTACCCACGTTGTTAACCCACTCGCATAACATCATCCCATCCTTTTAGC 65  
 O.mykiss ATGATT...ACCCTAATTACCCACGTTATTAATCCACTAGCATAACATTGTACCCATTCTGTTAGC 62  
 Consensus atgatt accctaattacccacgtt ttaa cact gcatacat t ccat ct ttage

s.t.caspius AGTTGCTTTCTCACCCTACTCGAACGAAAAGTCCTTGGGTATATACAACCTCGAAAAGGACCAA 130  
 O.mykiss AGTTGCTTTCTCACCCTACTTGAACGAAAAGTCCTTGGGTACATGCAACTTCGAAAAGGGCCCA 127  
 Consensus agttgctttctcaccctact gaacgaaaagtccttgggta at caacttcgaaaagg cc a

s.t.caspius ACATCGTCGGTCCATACGGATTACTTCAACCTATCGCGACGGCCTAAAACCTATTTATTAAGAA 195  
 O.mykiss ACATCGTCGGCCCCATACGGACTACTACAACCCATCGCAGACGGCCTAAAACCTATTTATTAAGAA 192  
 Consensus acatcgtcgg cc tacgga tact caacc atcgc gacggcctaaaactatttattaagaa

s.t.caspius CCAATCCGACCAATCCACCTCTCCACCTTCTATTTCTCGCTACACCTATACTTGCCTTCAACT 260  
 O.mykiss CCGGTTTCGACCTTCCACCTCTTCAACCTTCTATTTCTCGCTACACCCATACTTGCCTTCAACT 257  
 Consensus cc gt cgacc tccacctc tc cctt ctatttctcgtacacc atacttgcct acact

s.t.caspius CGCACTAACCTATGAGCCCCATACCCATCCCTACCCCAATTACGGACCTAAATCTAGGGGTAC 325  
 O.mykiss TGCACCTACTCTATGAGCCCCATACCTATCCCTACCTGTTACAGATCTTAACCTCGGAGTAC 322  
 Consensus gcaact ac ctatgagccc atacc at cc tacc ttac ga ct aa ct gg gtac

s.t.caspius TATTTATCTCGCACTCTCCAGCCTGGCCGTGATTCTATTTTAGGCTCAGGATGAGCTTCAAAAT 390  
 O.mykiss TATTTGACTTGCACCTATCCAGCCTGGCCGTGATTCTATTTTAGGCTCAGGATGGCATCAAAAT 387  
 Consensus tattt t ct gcaact tccagcctggccgtgattctattttagg tcaggatg gc taaa

s.t.caspius TCCAAATACGCTCTAATTGGAGCTCTACGAGCAGTAGCACAACCATCTCCTACGAAGTTAGCCT 455  
 O.mykiss TCCAAATACGCTTAAATTGGAGCCCTCCGGCAGTAGCACAACCATTCTCCTACGAAGTTAGCCT 452  
 Consensus tccaaatagct taattggagc ct cg gcaact gcacaaacat tctacgaagttagcct

s.t.caspius AGGACTAATCTTACTCGGCGTAATTATCTTTACAGGAGGATTTACTTCAAACCTTCAACGTAG 520  
 O.mykiss AGGCTTCACTTACTCAGCGTAATTATCATCACAGGAGGATTTACTTCAAACCTTCAACGTAG 517  
 Consensus agg t atcttactc gcgtaattatc t acaggaggatttac ct caaacctt aacgtag

s.t.caspius CCCAAGAAAGCATCTGACTACTCGTACCAGCCTGACCCTTGCCGCCATATGCTATATTCTACT 585  
 O.mykiss CCCAAGAAAGCATCTGACTACTCGTCCCGCCTGACCCTTGCCGCCATATGATACATTCTACT 582  
 Consensus cccaagaaagcatctgactactcgt cc gctgacc cttgcccgcataatg ta atttctac

s.t.caspius CTAGCTGAAACAAACCGCGCACCTTTGACCTCACAGAAGGAGAATCAGAGTTAGTCTCCGGGTT 650  
 O.mykiss CTCGCCGAGACAAACCGTGCACCTTTGACCTCACAGAAGGAGAGTCAGAAATTAGTCTCCGGATT 647  
 Consensus ct gc ga acaaaccg gcaccctttgacctcacagaaggaga tcaga ttagtctccgg tt

s.t.caspius TAATGTAGAATAACGCCGGAGGGCCATTGCCCTCTTCTTTCTAGCCGAATACGCTAATATCCTTC 715  
 O.mykiss CAATGTAGAATAAGCTGGAGGGCCCTTGGCCCTATTTTCTAGCCGAATAAGCTAATATCCTTC 712  
 Consensus aatgtagaata gc ggagggcc tt gccct tt tt ctagccgaata gctaataatccttc

s.t.caspius TAATAAATACACTCTCGACCATTTTATTCTTAGGCGCATCCACATCCCCTCCCTCCCGAATTA 780  
 O.mykiss TAATAAATACGCTCTCAGCGCTTCTATTCTTAGGCGCATCCACATCCCCTGCTTCCCTGAACTA 777  
 Consensus taataaatac ctct cc t tatt ttaggcgcataccacatccc gc tccc gaa ta

s.t.caspius ACAGCCGTAAATCTAATAACAAGGCCGCCCTCCTTCCGTTGTATTTTTATGAGTACGAGCCTC 845  
 O.mykiss ACTGCCCTAAACCTAATAACGAAGGCCGCCCTCCTCCTCGTTGTATTTTTATGAGTACGAGCTTC 842  
 Consensus ac gcc taaa ctaataac aaagccgccctcct tccgttgtatTTTTATGAGTACGAGCCTC

s.t.caspius CTACCCCGGATTTCGATACGACCACTCATAACCTTAGTTTGAAAACTTCTTACCTTTAACAC 910  
 O.mykiss CTACCCCGGATTTCGGTATGATCAACTCATAACCTTAGTTTGAAAACTTCTTACCTTTAACAC 907  
 Consensus ctacc ccgatttcg ta ga ca ctacatac ttagtttg aaaa cttc tacc t ac c

s.t.caspius TAGCTCTTGTCTGTGGCACCTAGCGCTTCCAACCGCAACAGCAGGCCTCCCCCTCA..... 969  
 O.mykiss TGGCCCTTGTACTATGACATTTAGCACTTCCCATCGCACTAGCAGGCCTCCCCCTCAGCTTTA 971  
 Consensus t gc cttgt ct tg ca tagc cttcc a cgca agcaggcctcccc

s.t.caspius	Max score	Total score	Query cover	E value	Ident	Accession
O.mykiss	1035	1035	99%	0.0	86%	59793

**Fig 4:** NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Oncorhynchus mykiss* (O. mykiss) were analysed with DNAMAN program and NCBI Network system. There were 126 NPs and 86% similarity between both sequences.

s.t.caspius	MITTLITHVVNPLAYIIPILLAVAFLLERKVLGYIQLRKGPNIVGPYGLLQPIADGLK	60
s.t.trutta	MITTLITHVVNPLAYIIPILLAVAFLLERKVLGYIQLRKGPNIVGPYGLLQPIADGLK	60
Consensus	mittlithvvnplayiipillavaflltllerkvlgylqlrkgpnivgpygllqpiadgk	
s.t.caspius	LFIKEPVRPSTSSPFLFLATPILALTLALTLPAPIPIYPITDLNLGVLFILALSSLAVY	119
s.t.trutta	LFIKEPVRPSTSSPFLFLATPILALTLALTLPAPIPIYPITDLNLGVLFILALSSLAVY	120
Consensus	lfikepvrpstsspflflatpilaltlaltl apipipypitdlnlglvlfilalsslavy	
s.t.caspius	SILGSGASNSKYALIGALRAVAQTISYEVSGLLILLGVIIFTGGFTLQTFNVAQESILLV	179
s.t.trutta	SILGSGASNSKYALIGALRAVAQTISYEVSGLLILLSVVIIFTGGFTLQTFNVAQESILLV	180
Consensus	silgsgasnskyaligalravaqtisyevsvglill viiftggftlqtfnvaqesillv	
s.t.caspius	PAPLAAIWYISTLAETNRAPFDLTEGESELVSGFNVEYAGGPFALFFLAEYANILLINTL	239
s.t.trutta	PAPLAAIWYISTLAETNRAPFDLTEGESELVSGFNVEYAGGPFALFFLAEYANILLINTL	240
Consensus	paplaaiwyistlaetnrpfdltegeselvsgfnveyaggpfalfflaeyanillintl	
s.t.caspius	STILFLGASHIPALPELTAVNLITKAALLSVVFLVRASYPRFRYDQLIHLVKNFLPLTLA	299
s.t.trutta	STILFLGASHIPALPELTAVNLITKAALLSVVFLVRASYPRFRYDQLIHLVKSFLPLTLA	300
Consensus	stilflgashipa peltavnlitkaallsvvflvrasyprfrydqlihvk flpltla	
s.t.caspius	LVLWHLALPTATAGLPPS	317
s.t.trutta	LVLWHLALPTATAGLPPQ	317
Consensus	lvl hlalptataglpp	

**Fig 5:** The amino acid sequences of NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Salmo trutta trutta* (S.t. trutta) with DNAMAN program. There were 3 SNPs between both sequences.

s.t.caspius	MITTLITHVVNPLAYIIPILLAVAFLLERKVLGYIQLRKGPNIVGPYGLLQPIADGLK	60
s.salar	MITTLITHIINPLAYIIPVLLAVAFLLERKVLGYMQLRKGPNIVGPYGLLQPIADGLK	60
Consensus	m ttlith nplayi p llavaflltllerkvlgylqlrkgpnivgpygllqpiadgk	
s.t.caspius	LFIKEPVRPSTSSPFLFLATPILALTLALTLPAPIPIYPITDLNLGVLFILALSSLAVYS	120
s.salar	LFIKEPVRPSTSSPFLFLATPILALTLALTLPAPMIPYPITDLNLGVLFVILALSSLAVYS	120
Consensus	lfikepvrpstsspflflatpilaltlaltlap pipypitdlnlglvlf ilalsslavys	
s.t.caspius	ILGSGASNSKYALIGALRAVAQTISYEVSGLLILLGVIIFTGGFTLQTFNVAQESILLVP	180
s.salar	ILGSGASNSKYALIGALRAVAQTISYEVSGLLILLSVVIIFTGGFTLQTFNVAQESILLVP	180
Consensus	ilgsgasnskyaligalravaqtisyevsvglill viiftggftlqtfnvaqesillvp	
s.t.caspius	APLAAIWYISTLAETNRAPFDLTEGESELVSGFNVEYAGGPFALFFLAEYANILLINTLS	240
s.salar	APLAAIWYISTLAETNRAPFDLTEGESELVSGFNVEYAGGPFALFFLAEYANILLMINTLS	240
Consensus	aplaaiwyistlaetnrpfdltegeselvsgfnveyaggpfalfflaeyanill ntlsl	
s.t.caspius	TILFLGASHIPALPELTAVNLITKAALLSVVFLVRASYPRFRYDQLIHLVKNFLPLTLAL	300
s.salar	AIFLFLGASHIPALPELTAVNLITKAALLSVVFLVRASYPRFRYDQLIHLVKSFLPLTLAL	300
Consensus	ilflgashipa peltavnlitkaallsvvflvrasyprfrydqlihvk flpltlal	
s.t.caspius	VLWHLALPTATAGLPPS	317
s.salar	VLWHLALPTAMAGLPPQ	316
Consensus	vl hlalpta aglpp	

**Fig 6:** The amino acid sequences of NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Salmo salar* (S. salar) with DNAMAN program. There were 14 SNPs between both sequences.



s.t.caspius	MITTLITHVWNPLAYIIPILLAVAFLLERKVLGYIQLRKGNIVGPYGLLOPIADGLK	60
O.mykiss	.MITTLITHVINPLAYIVPILLAVAFLLERKVLGYMQLRKGNIVGPYGLLOPIADGLK	59
Consensus	tlithv nplayi pillavaflltllerkvlg y qlrkgpnivgpygllqpiadgk	
s.t.caspius	LFIKEPVRPSTSSPFLFLATPILALTLALTLAPIPIYPITDNLGVLFILALSSLAVYS	120
O.mykiss	LFIKEPVRPSTSSPFLFLATPILALTLALTLAPIPIYPVITDNLGVLFVILALSSLAVYS	119
Consensus	lfikepvrpstsspflflatpilalaltlaltlapipipyp tdnlgvlf lalsslavys	
s.t.caspius	ILGSGASNSKYALIGALRAVAQTISYEVSGLLILLGVIIITGGFTLQTFNVAQESILLV	179
O.mykiss	ILGSGWASNSKYALIGALRAVAQTISYEVSGLLILLSVIIITGGFTLQTFNVAQESILLV	179
Consensus	ilgsg asnskyaligalravaqtisyevs lglill vii tggftlqtfnvaquesillv	
s.t.caspius	PAPLAAIWIYSTLAETNRAPFDLTEGESELVSGFNVEYAGGPFALFFLAEYANILLINTL	239
O.mykiss	PAPLAAI.YIYSTLAETNRAPFDLTEGESELVSGFNVEYAGGPFALFFLAEYANILLINTL	238
Consensus	paplaai yistlaetnrappfdltegeselvsgfnveyaggpfalfflaeyanillintl	
s.t.caspius	STILFLGASHIPALPELTAVNLITKAALLSVVFLVRASYPRFRYDQLIHLVKN.FLPLTL	298
O.mykiss	SAVILFLGASHIPAFPELTALNLITKAALLSVVFLVRASYPRFRYDQLIHLVWKSFLPLTL	298
Consensus	s lflgashipa pelta nlitkaallsvvflvrasyprfrydqlihlv flpltl	
s.t.caspius	ALVLWHLALPTATAGLPPS	317
O.mykiss	ALVL.HLALPIALAGLPPQ	316
Consensus	alvl hlalp a aglpp	

**Fig 7:** The amino acid sequences of NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Oncorhynchus mykiss* (O. mykiss) were analysed with DNAMAN program. There were 17 SNPs between both sequences.

#### 4. Discussion

##### 4.1 Why phylogenetic analysis of *Salmo trutta caspius* is important?

*Salmonid* species populations are native of some of the rivers draining to the Mediterranean and Black Sea (at least in upper Danube drainage) and the Caspian Sea (at least in upper Volga drainage). List Category and Criteria (red list) of *Salmo trutta* species was reported [3] which is a widespread species. However, anadromous part of populations (sea trout) and many lacustrine stocks have in many cases markedly declined because of pollution (and possibly from impacts from salmon farming). The phylogeographic structure is almost destroyed by stocking. However Freyhof [3] identified different species on the basis of morphological characters, but here we aimed to identify different species of *Salmonids* such as *Salmo trutta trutta*, *Salmo salar* and *Oncorhynchus mykiss* by comparing their NADH 1 gene. In other species of *Salmonids* like *Salmo trutta fario*, full length of their mitochondrial genome, specially their NADH 1 gene have been reported, but here we randomly selected *Salmo trutta trutta*, *Salmo salar* and *Oncorhynchus mykiss*, because the rate of homology between them is high.

##### 4.2 Why NADH 1 gene were used for phylogenetic analysis?

In this study we used NADH 1 gene for analysis of phylogenetic of *Salmonids*. However in salmonids phylogenetic have been reconstructed from morphology, physiology, ontogeny, DNA-DNA hybridization, protein electrophoretic mobility variation, karyology, DNA polymorphism and sequence analysis [1, 2, 8, 11, 13, 21-26]. Regarding NADH dehydrogenase, its genes have been

examined previously in *Salmonid* species and their sequences have been deposited in GenBank (Accession nos. U28345, U28364, U28365, U28366 and etc.). In this study we sequenced full length of NADH dehydrogenase 1 in *Salmo trutta caspius* for making it possible to examine the relationship among *Salmo trutta caspius*, *Salmo trutta trutta*, *Salmo salar* and *Oncorhynchus mykiss*. For getting good quality, the specimens were analysed by DNAMAN program and NCBI Network system. In this regards, we selected 20 samples from *Salmo trutta caspius*. After sequencing of NADH 1 gene, they were analysed within and between sequences, and we did not found any variation between them, so we selected one sequence from examined samples for comparing with *Salmo trutta trutta*, *Salmo salar* and *Oncorhynchus mykiss* populations.

##### 4.3 The situation of SNPs between sequences of NADH1 in *Salmo trutta caspius*, *Salmo trutta* and *Oncorhynchus mykiss*

Single nucleotide polymorphism (SNP) is a suitable technique for analysis of NADH 1 gene sequences, because the homology between sequences was high, so in this study we aimed for finding single nucleotide variations that were between these different sequences from different species, from the first to the last nucleotide of NADH 1 gene. According to DNAMAN program analysis, when comparing NADH1 gene, 23 SNPs were found between *Salmo trutta caspius* and *Salmo trutta trutta* for, 67 SNPs between *Salmo trutta caspius* and *Salmo salar*, and 126 SNPs between *Salmo trutta caspius* and *Oncorhynchus mykiss*. Amino acid sequences also were determined by DNAMAN program, according to its program settings, and between *Salmo trutta caspius* and *Salmo trutta*

*trutta* 3 SNPs, between *Salmo trutta caspius* and *Salmo salar* 14 SNPs, and between *Salmo trutta caspius* and *Oncorhynchus mykiss* 17 SNPs were observed. The situations of located mutations in nucleotides showed that there were not any single mutation on the start codon like ATG for three species cited above. Moreover, amino acid sequences also were studied and subjected to analysis by DNAMAN and its result showed three single mutations: G to S (Glycine to Serine), N to S (Asparagine to Serine) and S to Q (Serine to Glycine). *Salmo trutta caspius* and *Salmo salar* also showed mutations of I to V, V to I, V to I, I to V, I to M, I to M, I to V G to S, I to M, L to F, N to S and I to M and T to M respectively. *Salmo trutta caspius* and *Oncorhynchus mykiss* also were observed that have I to M, T to I, V to I, I to V, I to M, I to V, I to V, G to S, F to I, T to A, I to V, L to F, V to L, K to W, N to K, T to I, T to L, S to Q mutations.

According to the results of comparison between *Salmo trutta caspius* and *Salmo trutta trutta*, there were not any start codon like M (Methionine), but between *Salmo trutta caspius* and *Salmo salar* we found five M (Methionine) that were mutated from NADH 1 gene of *Oncorhynchus mykiss* to *Salmo salar*. In *Salmo trutta caspius* and *Oncorhynchus mykiss* we also found two Ms (Methionines) that mutated from NADH 1 gene of *Salmo trutta caspius* to *Oncorhynchus mykiss*. Hence, except for *Salmo trutta trutta*, both species of *Salmo salar* and *Oncorhynchus mykiss* had start codon mutations. At the level of nucleotides, we observed single nucleotide mutations between *Salmonids* of cited species. The percent of homology of *Salmo trutta caspius* was 98% with *Salmo trutta trutta*, 92% with *Salmo salar* and 86% with *Oncorhynchus mykiss* (Figures 4-6). According to those results, we concluded that *Salmo trutta caspius* has a higher homology with *Salmo trutta trutta* compared to *Salmo salar* and *Oncorhynchus mykiss* respectively. The sequence from start codon to stop codon is called the reading frame; and since synthesis of all polypeptide chains in eukaryotic cells begins with amino acid methionine, mutations on this region is very important for the organism. According to Figures 4-7, similarity and variety of sequences of NADH 1 gene between salmon species that cited in this study is important because Berg <sup>[2]</sup> had reported that *Salmo trutta* species originated from Atlantic Ocean and had migrated to White Sea and then left to Russia by the rout of Caspian Sea. Hence *Salmo trutta caspius* originated from *Salmo trutta* by morphological documents, however had reported between and within high homology on the *Salmonid* species such as *Salmo trutta fario* and *Salmo trutta caspius* when based to 16S rRNA gene, cytochrome C oxidase, ATPase subunit 6 and cytochrome b for population genetics <sup>[5-7, 9, 15-19]</sup>. Finally, we concluded that based on the sequence of NADH 1 gene, there is SNPs between *Salmo trutta caspius* and other *Salmonid* species, but in order to get the exact results about the rate of homology between salmon species, we have to study the full length of mitochondrial gene and also use other molecular techniques such as microsatellites and RFLP, RAPD.

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