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Studies of Cytochrome b nucleotide sequence variation in the *Salmo trutta fario*

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ABSTRACT

The cytochrome b gene in the *salmo trutta fario* has been sequenced and characterized and deposited in GeneBank, Accession Number (JN995186), the gene of cytochrome b approximately 1.2 kb. and consists of one exon from first to end of the gene, as found for all of salmonids including, *salmo salar*, *salmo trutta caspius*, *salmo trutta fario* that reported in GeneBank. There were the first to end of gene repetitive regions C-G that was unique for cytochrome b gene. At this study DNA extracted from muscles of *salmo trutta fario,* after running PCR on the gel, the PCR products purify and sequenced. The fragments aligned with BLAST Network system, the results are shown there were high homology between solmonids, the rate of homology between *salmo salar* 93%, *salmo trutta caspius* almost 95% and *salmo fario* (was reported in GeneBank) 93%. However, the homology of sequences were very high, but the shape of *salmo trutta fario* and other salmonids different, *salmo trutta fario* has red to purple color dots on the skin but regards *salmo salar* and *salmo trutta caspius* have grey to black dots colors on the skin.

Keywords: *Salmo trutta fario*, cytochrome b gene, sequence variation.

INTRODUCTION

Salmonids constitute one of the most manipulated fish in temperate countries. Moreover, Salmonid fishes, and specially the *salmo trutta fario*. *Salmo trutta fario* is a native salmonid from Eurasia to North Africa. There are natural distribution it exhibits an extreme phenotypic diversity and a considerable life history variation within geographical regions, including specialization for anadromous, fluviatile and lacustrine ecological modes of life (trutta, fario and lacustris, are the subspecies names currently assigned in the literature to fish possessing these specializations, respectively) (Behnke, 1972; Hamilton *et al*., 1989; Hindar *et al*., 1991). Present there are studies regard biological activities on the *salmo trutta fario*, including, marker genetics that contains microsatellites, alozymes which along with LDH5 and FBP1 (Poteaux 1995; Guyomard 1989) and mitochondrion DNA studies (Bernatchez *et al*., 1992).The using of DNA techniques and molecular researches for identification and characterization of fish for increasing production in them. (Gil,1992 and Rasmussen and Morrissey, 2008). In related to, we studied regard mitochondrial DNA in *salmo trutta fario*. In general, mtDNA targeted methods have predominated in such studies, because of the general robustness and higher cellular copy number of mtDNA compared with nDNA (Mackie *et al*., 199). Cytochrome b is a unique and essential required by cells to maintain aerobic metabolism, that proteins which contribute a function to the overall role of the mitochondrial encoded by mitochondrial DNA (mtDNA). In this study, we used PCR and direct

sequencing techniques to compare Cytochrome b DNA sequence variation observed among morphologically distinct *salmo trutta fario* and other salmonid populations by analyzing segments of coding genes, the cytochrome b in related to fragments of the mitochondrial control region specially cytochrome b gene studied by Bernatchez *et al*. (1992). This allowed to us that discuss the congruence in phylogenetic relationships among *salmo trutta fario* populations inferred from the analysis of coding and noncoding regions of the cytochrome b. because there were reports 1293 bp. length in *salmo salar* and there was not any report regard full length of the cytochrome b gene in GeneBank. With align of sequences we observed high homology between sequences.

MATERIALS AND METHODS

Sample collections: The samples of *salmo trutta fario* were collected from Rivers of Tonekabon –Iran. These samples had three ages old that collected on July of 2011. The originated of samples from bloods and muscles of fishes.

DNA isolation:

Total cellular DNA was isolated from powdered tissue has taken from muscle body following described by Sambrook *et al*., (1998). Briefly, tissue was digested in a digestion buffer, containing; (100 mM NaCl, 10 mM Tris pH 8.0, 25 rnM EDTA pH 8.0, 0.5% sodium dodecyl sulphate (SDS), 0.1 mg/ml proteinase K) for 18 hours at 50°C. Samples were then extracted with an equal volume of phenol-chloroform-isoamyl alcohol (24:25: 1).Vortexed 10 s then centrifuged at 3000 x g for 5 min at room temperature. DNA was precipitated overnight at 4°C with 1/2 vol 7.5 M ammonium acetate and 2 vol 100% ethanol. Then DNA solved in TE buffer (10 rnM Tris-CI, 1rnM EDTA) pH 8.0 and incubating at 37°C for 1 hour with 0.1% SDS. DNA samples were extracted again with an equal volume of phenol/ chlorofom / isoamyl alcohol and precipitated as described above. Upon the final resuspension in TE buffer pH 8.0. Samples were stored at-20°C until analysis. Then the DNA genomics amplified cytochrome b gene was separated by 1.5 % agarose gel electrophoresis. After electrophoresis, the DNA full length was visualized ethidium bromide and then was taken photos by gel DOC Bio RAD Company.

Designing of primers:

Primers designed to specifically amplify the cytochrome b gene based on conserved sequences from regions identified by the alignment of all the available sequences data from several salmonid species. These primers can amplified from first to end of cytochrome b gene including 1191 bp. These primers including:

Forward Primer 5' GACTTGAAAAACCACCGTTG 3'

Reverse Primer 5' CTCCGATCTCCGGATTACAAGAC 3'

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 (2.5mM each), 5 µl 10X ChromTaq Assay buffer, 0.5 µl ChromTaq enzyme (3U/µl), Water 37.5 µl, in a total volume, 50 µl. 94° of 5 min, 35 cycles of 94°C 30 Sec., 55°C 30 Sec., and 72°C 1 min. Two to ten µl of each PCR reaction were run on 1.5% agarose gels in TAE buffer containing ethidium bromide. One µl 500bp, 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.

Sequencing of the cytochrome b gene in *salmo trutta fario*:

For sequencing of cytochrome b gene we designed one set of primer, that process of the sequencing including:

PCR Purification: Amplified PCR product was purified using QIAquick PCR Purification Kit Protocol:
A. Added 5 volumes of Buffer PB to 1 volume of the PCR sample and mixed. **B**. Placed a QIAquick spin column in a provided 2 ml collection tube. **C**. Centrifuged at 8000 rpm for 30–60 s. **D**. Discarded flow-through. Placed the QIAquick column back into the same tube. **E**. Washed with 0.75 ml Buffer PE to the QIAquick column and centrifuged for 30–60 s. **F**. Discarded the flow-through and placed the QIAquick column back in the same tube. **G**. Centrifuged the column for an additional 1 min at maximum speed. **H**. Placed QIAquick column in a clean 1.5 ml microcentrifuge tube. **K**. To elute PCR product, added 40 µl of B H2O to the center of the QIAquick membrane and centrifuged the column for 1 min.

Sequencing of Amplified Cytochrome b gene:

Sequencing was performed along with the Forward and reverse primers in ABI 3730XL high throughput sequencer machine. Forward and reverse sequences were assembled and edited.

RESULTS

The ability of designed primers sets to detect of *salmo salar, salmo trutta* and *salmo trutta*. The amplified full length of gene cytochrome b had 1191 bp.

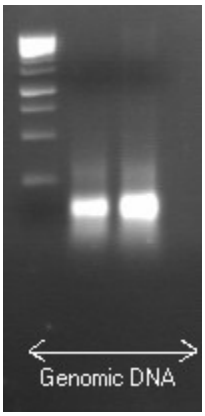

Fig.1: Total genomic DNA in *salmo trutta fario.*

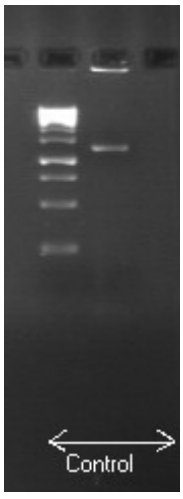

Fig. 2: PCR amplification of high quality *salmo trutta fario* using the primers of designed Samples were separated by electrophoresis in 1.5% gel electrophoresis. M. Size marker 1000bp.

Comparison of the cytochrome b in the *Salmo trutta fario* with other salmons:

In related to, compared between cytochrome b gene in the *Salmo trutta fario* and other Salmons, including *Salmo salar*,*salmo trutta*, *salmo trutta caspius* populations by the DNA gene program. Therefore the results are shown; there is high homology between sequences. In figure 3. A 99% DNA sequence similarity was observed, but there were five nucleotides differences from first of the two sequences and end of two sequences between sequence of *salmo trutta fario* and *salmo trutta caspius*. But regards *salmo salar* there was 93 percent homology between sequences. However there is some different the shape of salmons, *salmo trutta caspius* and *salmo salar* with *salmo trutta fario* but the analysis are shown homology was 93 percent, especially with *salmo salar*. Moreover these results innovated that *salmo trutta fario* in Iran is originated from *salmo salar* that the ancestor of *salmo trutta fario* had migrated from Atlantic Ocean to White Sea and then to Caspian Sea, because we collected samples has been connected from Rivers to Caspian Sea..

Sequences producing significant alignments:

Accession	Description	Max score	Max ident
JN995186.1	Salmo trutta fario cytochrome b (cytb) pseudogene, partial sequence; mitochon	2200	100%
FJ655773.1	Salmo trutta caspius cytochrome b (cytb) gene, partial cds; mitochondrial	1858	99%
FJ435622.1	Salmo trutta isolate SalmotruttaII tRNA-Glx (trnZ) gene, partial sequence; cytoc	1784	98%
FJ435621.1	Salmo trutta isolate SalmotruttaI tRNA-Glx (trnZ) gene, partial sequence; cytocl	1784	98%
AM910409.1	Salmo trutta trutta complete mitochondrial genome, specimen voucher Nor 00	1784	98%
EU492109.1	Salmo trutta trutta voucher SalTru_NS_02 cytochrome b (cytb) gene, complete	1775	98%
EU492108.1	Salmo trutta trutta voucher SalTru_NS_01 cytochrome b (cytb) gene, complete	1775	98%
EU492348.1	Salmo trutta trutta voucher NRM 52489 cytochrome b (cytb) gene, complete cd	1775	98%
FJ435623.1	Salmo trutta isolate SalmotruttaIII tRNA-Glx (trnZ) gene, partial sequence; cyto	1773	98%
EU492282.1	Salmo trutta trutta voucher NRM 51710 cytochrome b (cytb) gene, complete cd	1764	98%
EU492281.1	Salmo salar voucher NRM 53029 cytochrome b (cytb) gene, complete cds; mitoc	1764	98%
D58400.1	Salmo trutta mitochondrial gene for cytochrome b, partial cds	1762	98%
AF202033.1	Acantholingua orhidana cytochrome b gene, partial cds; mitochondrial gene for n	1701	96%
AF053590.1	Salmothymus ohridana cytochrome b (cytb) gene, mitochondrial gene encoding r	1701	96%
FJ608989.1	Salmo trutta haplotype 03 cytochrome b (cytb) gene, partial cds; mitochondrial	1581	99%
FJ608994.1	Salmo trutta haplotype 08 cytochrome b (cytb) gene, partial cds; mitochondrial	1578	99%
FJ608987.1	Salmo trutta haplotype 01 cytochrome b (cytb) gene, partial cds; mitochondrial	1576	99%
FJ608995.1	Salmo trutta haplotype 09 cytochrome b (cytb) gene, partial cds; mitochondrial	1572	99%
FJ608988.1	Salmo trutta haplotype 02 cytochrome b (cytb) gene, partial cds; mitochondrial	1570	99%
FJ608999.1	Salmo trutta haplotype 13 cytochrome b (cytb) gene, partial cds; mitochondrial	1563	99%
FJ608990.1	Salmo trutta haplotype 04 cytochrome b (cytb) gene, partial cds; mitochondrial	1555	99%
FJ608992.1	Salmo trutta haplotype 06 cytochrome b (cytb) gene, partial cds; mitochondrial	1550	99%
FJ608991.1	Salmo trutta haplotype 05 cytochrome b (cytb) gene, partial cds; mitochondrial	1550	99%
FJ608998.1	Salmo trutta haplotype 12 cytochrome b (cytb) gene, partial cds; mitochondrial	1546	99%
FJ608993.1	Salmo trutta haplotype 07 cytochrome b (cytb) gene, partial cds; mitochondrial	1544	99%
FJ608996.1	Salmo trutta haplotype 10 cytochrome b (cytb) gene, partial cds; mitochondrial	1526	99%
FJ608997.1	Salmo trutta haplotype 11 cytochrome b (cytb) gene, partial cds; mitochondrial	1520	98%
FJ435620.1	Salmo salar isolate SalmosalarIII tRNA-Glx (trnZ) gene, partial sequence; cytoch	1513	93%
FJ435618.1	Salmo salar isolate SalmosalarI tRNA-Glx (trnZ) gene, partial sequence; cytochrc	1507	93%

Figure 3: Comparison of sequences of the Cytochrome b gene between *salmo trutta fario* with other salmons. The maximum identity are shown, there are high homology between sequences.Regards *salmo trutta caspius* was 99% , *salmo trutta* 93% and *salmo salar* 93%.

Sequencing of cytochrome b gene:

For doing sequence from PCR products, we designed gene specific forward and reverse primers for synthesis of fragment of cytochrome b gene. Moreover was sequenced one fragment (figure 4), from first to end of the gene. That including:

(1) CGCCTCCGGTTTTCTCGTTACCCCTAGGGGCAGGTTCTTCCACCAGCTTTTATTTTCAG
CTCAGCCAGCCAAGGGGGCGAGAACTAGGAAGATAGTAAAGTAAATTACAGAGGCA
ACTTGACCGATGATGATAAATGGGTGTTCTACAGGTATCCCTCCAATTCAGGTGAGGA
TCAGTATGTCTGCTACTAGGGTTCAGAATAAGAATTGGGTTAGGGGGCGAAAGGTTA
GTCCGCGTTGCTTAGAGGTATGGAGGATGGGAACGACTATAAGGACCAGGATCGAGA
ATAAGAGGGCGAGTACTCCGCCCAGCTTATTAGGAATAGAGCGAAGGATTGCGTAGG
CGAATAGGAAGTATCATTCGGGCTTGATATGAGGCGGGGTGACTAGGGGGTTGGCAG
GCGTAAAATTGTCCGGGTCTCCGAGGAGGTTGGGTGCGAACAGAGCTAATGATGTTA
GGCCAAGTAGTATAGCTACGAATCCAAGGAGGTCTTTGTACGAGAAGTAGGGGTGGA
ATGAGATTTTATCGGCATCGGAGTTGATACCTGCTGGGTTATTAGAGCCGGTTTCATG
TAAAAATAGAAGGTGGAGTACTGTGGCAGCTGCAATAACGAATGGGAATAGGAAGTG
AAAGGCGAAAAATCGTGTTAGGGTGGCGTTGTCGACAGAAAATCCGCCTCAAATTCA
TTGTACAAGGGCGCCTCCAACGTATGGGACAGCGGAGAGAAGGTTTGTAATTACAGT
GGCTCCTCAGAAGGACATCTGTCCTCATGGAAGAACGTAGCCCACGAAGGCGGTTATT
ATAGTGAGAAGTAGCAGTACGACTCCGATATTTCAGGTTTCTTTATATAGGTAGGAAC
CATAGTAGAGTCCTCGGGCGATATGTATATAAATACAGATAAAGAAGAAAGATGCTC
CGTTAGCGTGAATGTTCCGGATGAGTCAGCCGTAGCTAACGTCTCGGCAAATGTGGCA
AACAGAGGAAAAGGCTGTTGAGATATCGGAGGTATAGTGTATGGCTAGGAAGAGCCC
GGTAGAATTTGGGTGGCTAGACACCAGCTAACATCAGAGCCCAAGTTGCCTCTGTAAT
TTACTTTACTATCTTCCTAGGTCTTGCCCCCTTGCGTCGATGAGCAATTTTTAGGAGGG
GGTGAGTTTTCGGAAGGTGGTTTTCCTAAATTTCCAATTGC (1191)

Fig. 4: The DNA sequencing of cytochrome b gene in the *salmo trutta fario*, from 1 to 1191 nucleotides. The major fragments have one exon from first to end of the gene. The fragment also contains GGGCC richness regions.

DISCUSSION

This study utilized partial mitochondrial encoded by cytochrome b gene, determine phylogenetic structure, identity areas high genetic diversity and endemism among aquatic taxa. The predictions also, are closely related morphological populations between species of *salmo trutta fario* in Iran and other places, for example in the different European regions. Comparing the estimated same morphology with this predictions assessment of the associated with designing primers against cytochrome b gene in *salmo trutta fario* however the salmonids were *salmo salar*, *salmo trutta caspius* and *salmo trutta*. The choice of a distant homology between outgroup of salmonids which could explain the apparent homology between *salmo salar* and *salmo trutta* at these nucleotide position, also was studied cytochrome b gene affected the rate of penetration and retention of ancestral sequence could have determined the fixation of genotypepic grouping specially cytochrome b gene in early times of differentiation between

salmo salar and *salmo trutta* populations from a common ancestor (Avise *et al*., 1993; Avise, Neigel & Arnold 1984) . It could also be evidence of a variation from the constant rate of molecular evolution (Gillespie 1984, 1986) or of selective processes (MacRae & Anderson 1988).Also, regarding cytochrome b in the *salmo trutta* and *salmo salar* the levels of variation detected among coding regions and sequence divergence estimates among variant that the cytochrome b gene segment represent the least variable region between sequences, however there were deletion or different nucleotides in the regions of segments, for example, in the 5' and 3' end segments of the control regions, the transition: transversion ratio was nearly 3:1(176) and deletions were found (Bernatchez *et al*., 1992; Brown *et al*., 1982 and 1993). The cytochrome b gene in salmonids have different length, in the *salmo salar* has, Accession Number, U12143.1 and EF584212, 1140 bp. (Philips *et al*., 1998). *salmo trutta caspius*, 1069 bp. Accession Number, FJ655773.1 (Jamshidi and Kalbasi, 2009) and *salmo trutta fario*, 310 bp. Accession Number, DQ394286.1, (De finizio *et al*., 2010) however the sequence of cytochrome b gene in the *salmo trutta caspius* was partial sequence. In *salmo trutta fario* were designed a pair of primers for amplify of full length, polymorphism was assessed at 9 samples and was observed on the gel a fragment of almost 1200 bp. then were purified PCR products and designed primers for the sequencing.These primers is expected to amplify one fragment 1 to 1191. Related to encompassing nucleotides of DNA in cytochrome b gene, the nucleotide-nucleotide BLAST search recovered numerous significant matches (>99% similarity) for cytochrome b gene sequences from other unionids. Empirical nucleotide base frequencies were biased towards thymine and adenine (27.7% A; 17.5% C; 29.9% G; 24.9% T).In related to, encompassing the fragments, positions of richness G-C, these characters are indicate unique at *salmo trutta fario* which almost like nucleotides situation in the *salmo salar.* This resulted also in higher frequency of the nucleotides GC, and GT, compared to the lower frequency of the nucleotides AT. These are fragments aligned with other sequences, especially *salmo salar* (accession no. U12143.1), *salmo trutta caspius* (accession number, FJ655773), these results are shown, there is high homology, 100% with *salmo salar* and almost 95% with *salmo trutta caspius*. Regards the *salmo trutta caspius* and *salmo salar* the results are shown however the variation between sequences was low but for typically *salmo trutta caspius* have grey to black dots color on the skin, but on the *salmo trutta fario* there is red to purple color on the skin. In other hand, the rate of growth in the *salmo trutta caspius*, *salmo salar* and *salmo trutta* more than *salmo trutta fario*, also there was high variation between sequences of the growth hormone gene in the *salmo trutta caspius, salmo salar* and *salmo trutta* with *salmo trutta fario*, (Rezaei *et al*., 2011, a, b) because the growth hormone gene relationship with paternal traits for increasing of growth body in the salmonids, (Gross *et al*.,1999; Nilsson *et al*., 2001) but regard of cytochrome b gene there were high homology between sequences.

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REFERENCES

Avise, J C.; Arnold, J. and Ball, RM. (1987). Intraspecific phylogeography: the mitochondria1 DNA bridge between population genetics and systematics. Annual Reviaus of

Ecology and Systematics, 18: 489-522.

Avise, JC.; Neigel JE. and Arnold, J. (1984). Demographic influences on mitochondria1 DNA lineage survivorship in animal populations. Journal of Molecular Evolution, 20: 99-105.

Bernatchez, L. and Danzmann, RG. (1993). Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of Brook cham *(Salvelinus fontinalis* Mitchill). Molecular Biology and Evolution, 10 (5): 1002-1014.

Brown, JR.; Beckenbach, AT. and Smith, MJ. (1993). Intraspecific DNA sequence variation of the mitochondria1 control region of white sturgeon (*Aciyenser transmontanus*). Molecular Biology and Evolution, 10: 326-341.

Brown, WM.; Prager, EM.; Wang, A. and Wilson, AC. (1982). Mitochondrial DNA sequences of primates: Tempo and mode of evolution. Journal of Molecular Ezrolntion, 18: 225-239.

Gil, L.A. (2007). PCR-based methods for fish and fishery products authentication. Trends Food Sci Technol., 18: 558-566.

Gross, R. and Nilsson, J. (1999). Restriction fragment length polymorphism at the growth hormone 1 gene in Atlantic salmon (*Salmo salar* L.) and its association with weight among the offspring of a hatchery stock. Aquacult., 173:73-80.

Guyomard, R. (1989). Diversite genetique de la truite commune, Bull Fr. Peche Piscic. 314: 118-135.

Hindar, K.; Jonsson, B.; Ryman, R. and Stdhl, G. (1991). Genetic relationships among landlocked, resident, and anadromous brown trout, Salmo trutta L. Heredity, 66: 83-91.

Mackie, IM.; Pryde, SE.; Gonzales-Sotelo, C.; Medina, I.; Perez-Martin, R.; Quinteiro, J.; Rey Mendez, M. and Rehbein H. (1999). Challenges in the identification of species of canned fish. TrendsFood Sci Technol., 10: 9-14.

MacRae, A F. and Anderson, W W. (1988). Evidence for non-neuhality of mitochondrial DNA haplotypes in Drosophila pseudoobscura. Genetics, 120: 485-494.

Nilsson, J.; Gross, R.; Asplund, T.; Dove, O.; Jansson, H.; Kelloniemi, J.; Löytynoja, A.; Nielsen, E.E.; Paaver, T.; Primmer, C.R.; Titov, S.; Vasemägi, A.; Veselov, A.; Ost, T. and Lumme, J. (2001). Matrilinear phylogeography of Atlantic salmon (Salmo salar L.) in Europe and postglacial colonization of the Baltic Sea area. Mol. Ecol., 10: 89-102

Poteaux, C. and Berrebe, P. (1997). Integerite genomique et repeuplements chez la truite commune du versant mediterranean, Bull Fr, Pecce Piscic. 344/ 345: 309-322.

Rasmussen, R S. and Morrissey, MT. (2008). DNA-based methods for the identification of commercial fish and seafood species. Compr Rev Food Sci Food Saf., 7:280-295.

Rezaei, A.; Akhshabi, SH. and Jamalzadeh, HR. (2011). Investigation and Identification *salmo trutta caspius* by the Growth Hormone type 1 (GH1) gene analogue. B. J. Biotech., 3: 76-82.

Rezaei, A.; Akhshabi, SH. and Jamalzadeh, HR. (2011). Sequencing and sequence analysis of Growth Hormone type 1 (GH1) gene homologue in the *salmo trutta caspius*. Adv. S. Biol., 3: 111-122.

Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. 2nd Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.