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# Investigation and characterization of the 16S rRNA gene in the *Salmo trutta fario* and *Salmo trutta caspius*

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## ABSTRACT

16S rRNA gene in the *salmo trutta fario* and *Salmo trutta caspius* has been polymerized, characterized and compared with within and between of *salmonids* species. The full lengths of 16S rRNA gene in species of salmons were sequenced approximately 1.7 kb and deposited in GenBank. We designed one pair of primer for amplification of 16S rRNA gene in species of *s.t.caspius* and *s. t. fario*. In present study, DNA extracted from muscles of *s. t. fario* and *s.t.caspius* for amplification of the 16S rRNA gene. After running PCR on the gel, against the PCR product had taken photo by Gel DOC. The results are shown there was high homology between *solmonids*, because we got PCR product around 1700bp equal of full length of 16S rRNA gene that reported in GenBank. However, the homology of PCR products were very high, but the shape of *s. t. fario* with *s.t.caspius* is different, *s.t.fario* has red to purple color dots on the skin but regards *salmo salar* and *s.t. caspius* have grey to black dots colors on the skin.

**Keywords**: *Salmo trutta caspius*, *Salmo trutta fario*, Mitochondrial genomic, 16S rRNA gene

## INTRODUCTION

*Salmo trutta* comprises several distinct as far as their classification as a species or subspecies is concerned (Berg, 1948; Giuffra *et al.*, 1994, Patarnello *et al.*,1994; Osinov and Bernatchez, 1995). *S. t. caspius* living in the sea and migrating widespread in a large number of water streams of the mountain area and around rivers of them. The present project, we studied on the phylogeny and evolution of *salmons*. So, despite in fact a large number of studies based on both the morphological (Nrden, 1961; Stearley, 1992; 1993) and molecular genetics data (Phillips *et al.* 1995; 1997; Kitano *et al.* 1997; Oohara *et al.* 1997; Crespi, *et al.*, 2003).

In the *salmonids* there were some single variation between and within them when compared by cytochrome b gene in *s. t. fario* (Rezaei *et al*., 2011) and growth hormone gene type I gene in *s. t. caspius* (Rezaei *et al*., 2011). However, with increasing level of nucleotide sequence variation can because of the rate of evolution of *salmonids*. Hence, studies on the maternal inheritance and higher mutation rates compared to those of nuclear genes, which mitochondrial DNA (mtDNA) has demonstrated to be valuable in molecular phylogenetic studies (Avise, 2004). The mitochondrial genomic has thirteen protein-coding genes, that contains 22 tRNA and 2 rRNA genes, the result of phylogenetic analysis is shown that there were 95.2% to 98.2% identity between them (Thompson *et al*., 1997; Clayton, 1982; 1991; Shadel and Clayton, 1997).

The mitochondrial 16S rRNA gene has been used to explore the phylogenetic relationships of fishes at different taxonomic levels (Orti and Meyer, 1997; Moyer *et al.*, 2004; Feng *et al.*, 2005; Li *et al*., 2008), mainly due to the fact that it is highly

conserved and has a slow evolution (Page and Holmes, 1998). In order to, 16S rRNA were sequenced and deposited in GenBank, Accession number (NC; 001960). According to (Hurst, C. D. 1999), who had reported, 16S rRNA gene by amplification of PCR in the Atlantic salmon (*Salmo salar*). Also Lubieniecki, K.P. (2012), were sequenced complete 16S rRNA gene in GenBank, Accession number, JQ390057, the result of analysis are shown, the rate of variation between *salmons* when compared with 16S rRNA gene is very low. The result of homology of 16S rRNA gene, with other *salmonids* was same too. The aim of our study is analyze the 16S rRNA gene in *s. t. fario* and *s. t. caspius* between *salmonids* species that reported in Genbank and within *s.t. caspius* and *s. t. fario* to evolutionary of descent of *salmonids.* In addition, the suitability of these markers for further population genetics and phylogenetic studies *salmonids* in Iran.

## MATERIALS AND METHODS

### Sample collection and DNA extraction

Samples from *s. t. caspius* and *s. t. fario* were collected from Rivers of Tonekabon-Iran. The specimens representing fragments of fins were fixed in 96% ethanol. Total DNA was extracted from fin tissue using the method described by Taggart *et al.* (1992), with minor modifications. Briefly, tissue was digested in a digestion buffer, containing; (100 mM NaCl, 10 mM Tris pH 8.0, 25 mM 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), the samples after centrifuged for 5 min in room temperature again was added purify ethanol(96%) for washing DNA and finally DNA solved in 50 µl TE buffer, then samples were stored at -20°C until using for PCR reaction.

### Designing of the primers:

According to among homology between 16S rRNA gene reported in GenBank, we designed one pair of primers for *S. t. caspius* and *S. t. fario*, these primers including:

Forward Primer: 5' CACCTCCCTTACACCGAGAA 3'
Reverse Primer 5' GCCGAGTTCCTTCTCTTCCT 3'

### The PCR programs:

The PCR reactin used 10 µg 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 buffer, 0.5 µl Taq enzyme (3U/µl), Water 37.5 µl, in a total volume, 50 µl. 95°C of 10 min, 30 cycles of 94°C for 30 Sec., 52.3ºC 30 Sec., and 72°C, 90 Sec. Two to ten µl of each PCR reaction were run on 1.5% agarose gel in TAE buffer containing ethidium bromide. One µl 500bp, DNA ladder (Gibco-BRL) was used as a size standard. Then DNA full length was visualized ethidium bromide and was taken photos by Gel DOC Bio RAD Company.

## RESULTS

Study of PCR factors for optimization, including, concentration template of DNA, annealing temperature, extension time were used. At present study, we isolated DNA by Kit, because by kit we got more than DNA and the quality of DNA was good (Fig. 1). For optimization of the PCR work we used gradient temperature from 50ºC to 60ºC. The gradient temperature were fixed in 52.3ºC, the other parameters of PCR

(DNA template, time of extension and number of cycle extension, from a pair of primer of 16S rRNA gene were optimised (Fig. 2). A fragment of approximately 1700bp amplified and showed by 1000bp size marker.

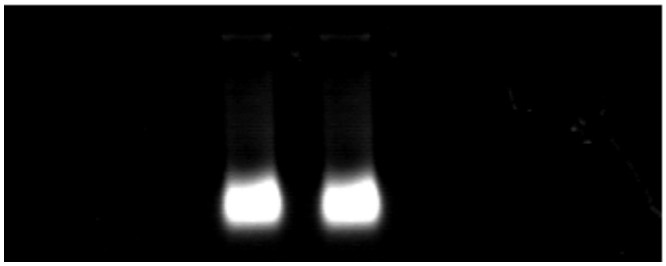

Fig. 1: Total genomic DNA in *s. t. fario* and *s .t. caspius.*

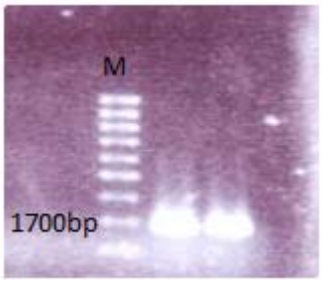


Fig. 2: PCR product of 16S rRNA gene from *s. t. fario* (left band) and *s.t.caspius* (right band). The results are shown, high homology between them. M: Size marker 1000bp.

## DISCUSSION

The mitochondrial genomic generally transferred maternal traits. Structural organization of the mitochondrial genes and noncoding regions are found in *salmonids* that consisting of 2 rRNAs, 22 tRNAs, and 13 protein-coding genes with noncoding and coding genes regions (Clayton, 1982; 1991; Shadel and Clayton,1997). These regions shows extensive variability across taxonomic groups and even among closely related species of other fishes (Clayton, 1982; 1991; Shadel and Clayton, 1997). Depending of phylogenetic analysis was supported by 16S ribosomal RNA gene. However, 16S rRNA gene was used for studies of phylogenic in the some *salmonids*, for example, *Salvelinus* and *Oncorhynchus* (Shadel and Clayton, 1997). The relationship between *salmonids* was also supported by previous molecular phylogeny studies based on two other genes contains, Growth hormone gene (GH1) in *s. t. caspius* and cytochrome b in *s.t. fario* (Rezaei and Akhshabi, 2011; Rezaei *et al*., 2011; Rezaei and Akhshabi, 2012; Rezaei *et al*., 2012; Rezaei, 2012a; Rezaei, 2012b). In our study, the individual gene supports polymorphism of *salmonids*. Phylogenetic analysis of 16S rRNA gene in *s. t. fario* and *s.t. caspius* shows that *salmonids* genus having same homology and least variation between them. Our data reveal a close relationship between *S. t. fario, s. t. caspius* and the clade formed by sea trout (*Salmo trutta trutta*) (Accession number; JQ390057.1) (Lubieniecki *et al.,* 2012) and the Atlantic salmon (*S. salar*) (Accession number; U12143.1)(Philips *et al.*, 1998). The resulting clade (*S. t. fario, s.t. caspius, s.t. trutta* and *s.salar*) are surprising, taking into consideration some characteristics of the life history and reproductive behaviour of these species (Bernatchez *et al.,* 1992; Brown *et al.,* 1982 and 1993; Avise *et al.*, 1993; Avise *et al.*, 1984). Furthermore, result of phenotypic are shown, *s. t. fario* and *s. t. caspius* have different shape, *s. t. fario* in hatchery trout has a bluish grey body colour and red spots are always observed in populations and no black strips but in *s.*

*salar*, *s. t. caspius* and *s. trutta*, more regular in shape, and less intensively pigmented. However, the shape and size of *s. t. fario* is different with *s. t. caspius* but the among of homology were high, hence we can concluded; 1. May be related to region of living them, because they are living Rivers of the North of Iran that connected to Caspian Sea; perhaps these species with themselves has been conjugated from many years ago. 2. However in this study showed between *s. t. fario* and *s. t. caspius* about 16S rRNA gene have been high homology, after sequencing of the full length of 16S rRNA gene and generally full length of mitochondrial genomic we can exactly discuss about among of relationship between *s.t. fario*, *s.t.caspius* and other *salmonids*. Also, we should more research and using other method genetic techniques for finding ancestor of *salmons* specially relationships between *salmonids*.

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## REFERENCES

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

Avise, J. C. (2004). Molecular markers, natural history and evolution. 2nd. Chapman and Hall, London, 511 pp.

Berg, L. S. (1948). Freshwater fishes of the USSR and adjacent countries. Zoological Institute Akademy Nauk Moscow USSR., 1(27):1.

Bernatchez, L., Guyomard, R., Bonhomme, F. (1992). DNA sequence variation of the mitochondrial control region among geographically and morphologically, remote European brown trout *Salmo trutta* populations. Molecular Ecology. 1:161 -1 73.

Brown, J.R., Beckenbach, A.T., Smith, M.J. (1993). Intraspecific DNA sequence variation of the mitochondria1 control region of white sturgeon *(Aciyenser transmontanus).* Molecular Biology and Evolution. 10: 326-341.

Brown, W.M., Prager, E.M., Wang, A., Wilson, A.C. (1982). Mitochondrial DNA sequences of primates: Tempo and mode of evolution. Journal of Molecular Ezrolntion. 18: 225-239.

Crespi, B. J., Fulton, M. J. (2004). Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny. Molecular Phylogenetics and Evolution. 31: 658–679.

Clayton, D.A. (1982). Replication of animal mitochondrial DNA. Cell. 28: 693–705.

Clayton, D.A.(1991). Nuclear gadgets in mitochondrial DNA replication and transcription.Trends Biochemistry Science. 16: 107–111.

Feng, Y., Jing, L., Peijun, X. J. (2005). Preliminary study on mitochondrial 16S rRNA gene sequences and phylogeny of flatfishes (Pleuro nectiformes). Chinese Journal of Oceanology and Limnology. 23: 335–339.

Giuffra, E., Bernatchez, L. Guyomard, R. (1994). Mitochondrial control region and protein coding genes sequence variation among phenotypic forms of Brown trout, Salmo trutta from Northern Italy. Molecular Ecology. 3: 161–172.

Hurst, C.D., Bartlett, S.E., Davidson,W.S., Bruce, J.J. (1999). The complete mitochondrial DNA sequence of the *Atlantic salmon*, (Salmo Salar). Gene. 239 (2): 237-242.

Kitano, T., Matsuoka, A, N., Saitou, N. (1997). Phylogenetic relationship of the genus Oncorhynchus species inferred from nuclear and mitochondrial markers. Genes & Genetic Systems. 72:25–34.

Li, J., Wang, X., Kong, X., Zhao, K., He, S., Mayden, R. L. (2008). Variation patterns of the mitochondrial16S rRNA gene with secondary structure constraints and their application to phylogeny of cyprininae fishes (Teleostei: Cypriniformes). Molecular Phylogenetics and Evolution.47: 472–487.

Lubieniecki, K.P., Saeed, D., So, M., Fujiki, K., Koop, B.F. Davidson,W.S. (2012). Evolution of *Salmoniformes* based on their mitochondrial genome. Unpublished.

Nrden, S. (1961). Comparative osteology of representative salmonid fishes, with particular reference to the Grayling (T. Arcticus) and its phylogeny. Journal of the Fisheries Research Board of Canada. 8: 679–79.

Oohara, I., Sawano, K., Okazaki, T. (1997). Mitochondrial DNA sequence analysis of the Masu Salmon Phylogeny in the genus Oncorhynchus. Molecular Phylogenetics and Evolution.7: 71–78.

Orti, G., Petry, P., Porto, J. A., Jegu, M. and Meyer, A. (1996) Patterns of nucleotide change in mitochondrial ribosomal RNA genes and the phylogeny of piranhas. Journal of Molecular Evolution.42: 169–182.

Osinov, A., Bernatchez, L. (1996). Atlantic and Danubian phylogenetic groupings of brown trout (Salmo trutta L.) complex: genetic divergence, evolution, and conservation. Journal of Ichthyology. 36: 762–786.

Page, R. D. M., Holmes, E. C. (1998). Molecular evolution: A phylogenetic approach. Blackwell Science, Oxford, pp. 172–227.

Phillips, R. B., Oakley, T. H. (1997). Phylogenetic relationships among the Salmonidae based on nuclear DNA and mitochondrial DNA sequences. 145–162.

Phillips, R. B., Sajdak, S. L., Domanico, M. J. (1995). Relationships among chars based on DNA sequences. Nordic Journal of Freshwater Research. 71: 378–391.

Patarnello, T., Bargelloni, L., Caldara, F., Colombo, L. (1994). Cytochrome b and 16S rRNA sequence variation in the Salmo trutta (Salmonidae, Teleostei) species complex. Molecular Phylogenetics and Evolution. 3: 69–74.

Rezaei, A. (2012a). Studies of Cytochrome b nucleotide sequence variation in the *Salmo trutta fario*. Egypt. Acad. J. Bio. Sci. 3(1):125-131.

Rezaei, A. (2012b). Studies of Cytochrome b nucleotide sequence variation in the *Salmo trutta fario*. Egypt. Acad. J. Biolog. Sci., 3(1):125-131.

Rezaei A., Akhshabi, S.H. (2012). Studies of cytochrome b protein modeling sequence in the *Salmo trutta fario* and comparing with other salmonids. Egypt. Acad. J. Biolog. Sci., 4(1):1-8.

Rezaei, A., Akhshabi S.H. (2011). Evolutionary Genetic Analysis of the cytochrome b gene variation in the *Salmo trutta fario* with other salmons. Egypt. Acad. J. biolog. Sci., 3(1):65–71.

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

Rezaei, A., Akhshabi S.H., Jamalzadeh H.R. (2012). The relation between growth hormone (GH) gene and Cytochrome b gene in three salmon types. Egypt. Acad. J. Biolog. Sci., 3(1):133–140.

Shadel, G.S., Clayton, D.A., 1997. Mitochondrial DNA maintenance invertebrates. Ann. Rev. Biochem. 66: 409–435.

Stearley, R. F., Smith, G. R. (1993). Phylogeny of the Pacific trouts and salmons (Oncorhynchus) and genera of the family Salmonidae. Transactions of the American Fisheries Society., 122: 1–33.

Stearly, R. F. (1992). Historical ecology of Salmoninae, with special reference to Oncorhynchus. pp.622–758. In: MAYDEN, R. L. (Ed.): Systematics, historical ecology, and North American freshwater fishes. Stanford University Press, Stanford, CA.

Thompson, J. D., Higgins, D. G., Gibson, T. J. (1994). CLUSTAL-W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research. 22: 4673–4680.

Taggart, J. B., Ferguson, A. (1991). Hyper variable mini-satellite DNA single locus probes for the *Atlantic salmon* (Salmo solar L) . Journal Fish Biology., 37: 991-993.