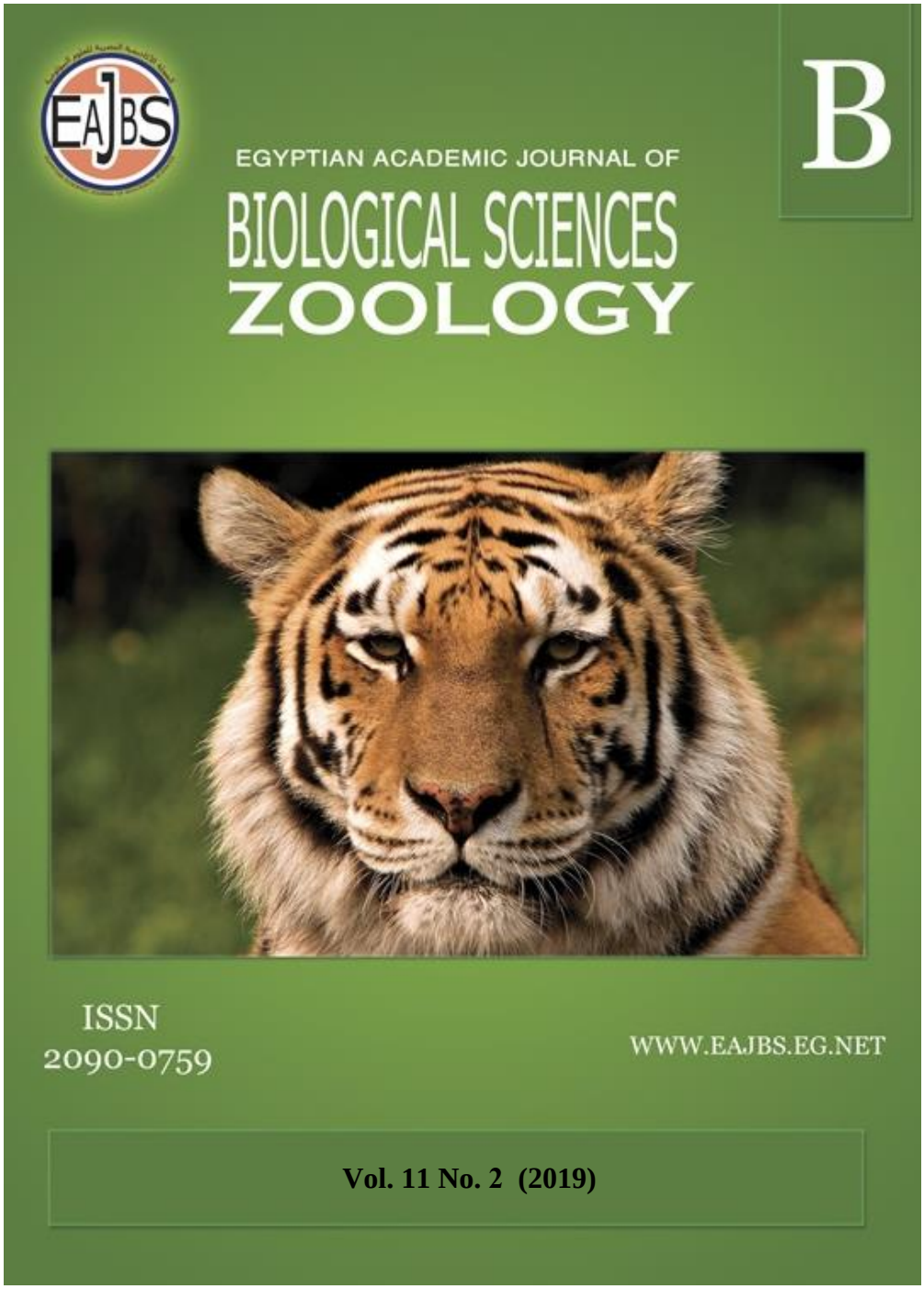

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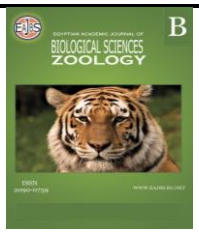

Sequencing Analysis of Mitochondrial Genomic and Relationship between *Salmo Trutta Fario* Populations in Iran

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ABSTRACT

In this study, phylogenetic and morphological studies of *Salmo trutta fario* isolate Persicus with partial and complete mitochondrial sequencing was carried out. The full length and partial sequencing (NADH 1 gene) were sequenced and deposited in GenBank accession numbers LC137015, LC137894, LC154931, LC1549281, and LC011387.1. Almost all meristic traits values were the same between *Salmo trutta fario* caught from the four regions Cheshmeh Kile, Jaj Roud, Ghasem Abad and Siahkal Roud. On the other hand, studies of molecular phylogenetic-based Maximum Parsimony between *Salmo trutta fario* isolate Persicus and another 50 nucleotide sequences of Salmonid species showed low variation between *Salmo trutta fario* (LC137015), *Salmo trutta caspius* (LC011387.1) and *Salmo Salar* (different accession numbers). Moreover, Maximum Composite Likelihood Estimate conducted was conducted using MEGA software version 7.0. According to that, rates of different transitional and transversional changes are shown. The resulting nucleotide frequencies are 29.76% (A), 26.34% (T/U), 24.24% (C) and 19.66% (G). The transition/transversion rate ratios are k1= 23.652 (purines) and k2 = 0.053 (pyrimidines). The analysis involved five nucleotide sequences. According to the lengths of branches, *salmo trutta fario* – Jaj Roud 1 (LC154929), *salmo trutta fario*-Jaj Roud 2 (LC154931.1), *salmo trutta fario*-Siahkal Roud (LC1549281), *salmo trutta fario*-Cheshmeh Kileh (LC137015), and *salmo trutta fario*-Ghasem Abad (LC137894) variety were different, respectively. Comparison of partial mitochondrial sequence in *Salmo trutta fario* for the four regions cited above showed that approximately 99% homology existed between them. In conclusion, the mitochondrial genome homology of *Salmo trutta fario* and other salmonids were high.

INTRODUCTION

Salmonidae species only have one family under the name of salmonids, which encompasses about 70 species. Salmonids can be divided into three groups including Salmoninae (*Salmo trutta caspius* and *Salmo trutta fario*), Chorine Chorine and Timalinae (Geraylings) (Bernatchez 2011). The scientific name of *salmo trutta fario* belongs to the family of salmonids (Salmonidae). They migrated for reproductive activity from one river to another that ultimately connected to the northwest of the Caspian Sea. Presumably, the ancestors of the migrated from the White Sea to the Caspian Sea through the Volga River. The body of the *salmo trutta* is mildly stretched and compressed from both sides and has two dorsal fins. They are seen on the surface of a red dot body that some fishes like X form having two races in spring and autumn. The fall migration occurs from late September to November, and the spring migration takes place in April and May. The fish attempt to lay eggs both in late autumn and early winter. In the present study, phylogenetic analysis of the *Salmo trutta fario* isolates Persicus mitochondrial genomes are compared with the inner group of *Salmo trutta fario* and an outer group of *Salmo trutta fario* with known salmon in throughput. Researchers have also investigated the connection between these groups, but the origin of this research is subject to Berg's theory that was proposed in 1948. According to his theory, about 11 to 14 thousand years ago, *salmo trutta* have migrated from the White Sea to the Caspian Sea, and have chosen the south of the Caspian Sea that are connected to the rivers of Iran. Subsequently, they found two split species *of Salmo trutta fario* and *Salmo trutta caspius*. *Salmo trutta fario* usually choose rivers leading to the Caspian Sea as their habitat and location for reproduction; they are widely characterized into suitable environments around the world, including North and South America, Australia, Asia, and South and East Africa (Rocha et al., 1994). Introduced Brown trout have established self-sustaining, wild populations in many introduced countries, especially in Iran, which they are found in rivers connected to the Caspian Sea from east to west. The aim of this study is to study other salmonids by conducting a phylogenetic study on *Salmo trutta fario* isolate Persicus intergroup and outergroup. Phylogenetic studies are important and critical for environment-living and aquaculture science. Hence, *Salmo trutta fario* is mentioned as a threatened species by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (www.iucnredlist.org). The aim of this research is to examine phylogenetic and genotypic studies. Regarding phenotypic factors, different phenotypes are produced to increase the capacities of genotypic factors (Stearns, 1989; Scheiner, 1993). Several studies have been performed in relation to the phenotypic traits of salmonid species (Thymallus thymallus, *Salmo salar m. sebago, Salmo trutta m. lacustris* and *Salvenilus alpines*,

Pakkasmaa et al., 1998; *Salmo trutta*, Oadri, 1959; İslam et al., 1973; Blanc et al., 1982, 1994; Skaala and Jørstad, 1988; Mezzera et al., 1997; Dyness et al., 1999; Pakkasmaa and Piironen, 2001; Agapova et al., 2002; Alexander and Adams, 2004; Aparicio et al., 2005; Keeley et al., 2005; Keeley et al., 2006; Bronte and Moore, 2007; Bud et al., 2009). Some phenotypic parameters that were compared are the spotting patterns in salmonid bodies including black and red spots below and above the lateral line, black and red spots on the fin adipose, and the total number of red and black spots (Aparico et al. 2005). On the other hand, differenced and genetic diversity were observed in the animal groups and between them ((Brown, 1980; Avise et al., 1979; Lansman et al., 1983a) and showed in a population of salmonids ((Zardoya et al., 1995). In general, mtDNA of 67 fish were sequenced and reported in (NCBI:

www.ncbi.nlm.nih.gov/PMGifs/Genomes/7898.html). On the other hand, Hurst et al. (1999) examined the sequence of mtDNA in *Atlantic Salmon* (Salmo salar), *Salmo alpinis*, and *Salmo fontinalis* (Doiron et al., 1999; 2000). Mitochondrial genomics and nuclear genomics are related to inheritance (maternal and paternal traits); although paternal traits may represent diversity, maternal traits lack recombination in their results. Therefore, maternal traits are usually the best parameter for phylogenetic studies especially in salmonid species. In Iran, both species of *Salmo trutta caspius* and *Salmo trutta fario* are topics in the phylogenetic study of salmonid fishes. They usually live in most of the rivers connected to the Caspian Sea (Armantrout, 1980; Saadati, 1977; Coad, 1979; Araghi, 1996; Abdoli, 1999; Kiabi et al., 1999: Afrayi et al., 2000; Abbasi et al., 2004; Vatandoost et al., 2008; Ghane, 2008; Kheyrandish, 2010; Kazancheev (1981). Nevertheless, studies on salmonid species ans essentially important in Iran. In the present study, we aimed to examine the phylogenetic and phenotypic studies between *salmo trutta fario* population in the major rivers of Iran and in comparison with other known salmonid species in other parts of the world.

MATERIALS AND METHODS

Salmo trutta fario Isolate Persicuas Samples:

In this study, one hundred samples were used from four regions including Cheshme Kileh-Tonekabon, Ghasem Abad, Siahkal Roud and Jaj Roud (twenty-five samples per region) (All of salmons were collected from the natural population in above-mentioned rivers). Phenotypical analysis was conducted according to standards typical of fishes. Total length, weight and number of spots in *salmo trutta fario* isolate Persicuas were measured. They were caught in fall 2015, which they were approximately three years old at that time. On the other hand, salmons were anesthetized, and after tagging their left side, they were photographed and the right pectoral fin was clipped, the whole fish was tagged and fixed in 96% ethanol.

Total DNA was extracted from fin tissue (around 5-6 grams of muscle was powdered by nitrogen solution in -180°C). Then, genomic DNA was extracted by phenol-chloroform and ethanol precipitation protocol (Sambrook et al., 1989).

Designing Primers and Sequencing of Mitochondrial Genome (Part and Full of Fragment):

According to other studies, *Salmo trutta fario* has high homology with other salmonid species like *Salmo salar* (NC_00861) and *Salmo trutta* (JQ390057), so we decided to choose the mtGenome of *Salmo salar* (NC_00861) for the purpose of designing the primers. Thirty-three pairs of primers were designed and selected for PCR reaction. Conserved regions were selected to place overlapping forward and reverse primers. Thereafter, the PCR product was sequenced by the sequencing machine.

Mitochondrial Sequencing Submission and Bioinformatics Analysis:

Sequencing was initially conducted using the forward PCR primer for full length and NADH1. A sequence of approximately 16665 and 900 to 1000 base pairs was obtained using these primers for full length and ND1. One full mitochondrial genome from *Salmo trutta fario* isolate Persicuas was deposited in the GenBank accession no. LC137015 and four sequences from the NADH 1 gene also from the four regions (*salmo trutta fario*-Jaj Roud 2 (LC154931.1) and *salmo trutta fario*-Siahkal Roud (LC1549281), *salmo trutta fario* (LC137015), *salmo trutta fario*-Siahkal Roud and *salmo trutta fario*-Ghasem Abad (LC137894). Multiple sequence alignment of the fifteen *Salmo trutta fario* isolate Persicus mtDNA sequences was generated using

DNAMAN software version 7 for diversity of nucleotides, UPMGA (Unweighted Pair Group Method with Arithmetic Mean) (Sokal and Sneath 1973) analysis to infer phylogeny based on pairwise genetic distance between individuals. Maximum Parsimony (MP) for analysis of neighbor-joining tree also were applied for this study.

RESULTS

Morphological Analysis:

In Table 1. Showed that the schematic of *salmo trutta fario* isolate Persicus from four regions of rivers in Iran(Cheshme kileh(A), Ghasem Abad(B), Siahkal Roud(C) and Jaj Roud(D). Phenotypic characterization was based on 17 meristic traits. The meristic traits examined were the red spots on the left side, pectoral, pelvic, dorsal, and anal fin soft rays, scales on lateral and above the line, scales between lateral line and fin fat, number of spines and soft rays in ventral fin, number of spines and soft rays in Pectoral fin. Soft rays of Caudal fin, Gill rakers, Gill filaments, Number of trunk vertebrate, Pyloric Coeca. For morphometric analysis, photographic images were made of the left side of each specimen. All specimens in this study placed on the landmark in the same position for photography with a digital camera. (Salavatian et al. 2011). On the other hand, Figure 1 showed mean value of morphometrically analysis of hundred samples of *salmo trutta fario* from four regions (Cheshme kileh, Ghasem Abad, Siahkal Roud and Jaj Roud) were done with a digital camera. Morphometrically analysis as follows; weight; 887.9±54.2 g, total length; 230.75±30.0 mm, fork length: 230.81±32.6 mm, standard length: 200±40.4 mm. Rearrangements of Sequences Evolutionary analysis Evolutionary analyses were conducted in UPGMA method of the pattern of 50 nucleotide sequences of salmonid species was inferred using a pre-computed tree file (Fig. 2). There were 16997 positions in the final dataset. According that were observed variety between subspecies of Iranian salmonid (black underline marked)such as *salmo trutta fario* isolate Persicus-Cheshme Kileh (LC137015), *salmo trutta fario* - Ghasem Abad (LC137894), *salmo trutta fario* -Jaj Roud 2 (LC154931), *salmo trutta fario* -Siahkal Roud (LC1549281) and *salmo trutta caspius* (LC011387.1). Figure 3. Each entry shows the probability of substitution (r) from one base (row) to another base (column) conducted in MEGA7. According that the sum of R-values is made equal to 100. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. The nucleotide frequencies are 29.76 %(A), 26.34% (T/U), 24.24% (C), and 19.66% (G). The transition/transversion rate ratios are k1= 23.652 (purines) and k2 = 0.053 (pyrimidines). The analysis involved 50 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing gaps and missing data were 705 positions in the final dataset. In Figure 4. Evolutionary relationships between under group of *salmo trutta fario* isolate Persicus used this study was inferred using the UPGMA method by MEGA Version 7. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved five nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were 705 positions in the final dataset. According to the length of branch *salmo trutta fario* -Jaj Roud 2(LC154931.1), *salmo trutta fario* -Siahkal Roud (LC1549281.1), *salmo trutta fario* Cheshme kileh (LC137015.1) and *salmo trutta fario salmo trutta fario* -Ghasem Abad (LC137894), variety were different respectively. Figure 5 showed five sequences of partial mitochondrial sequencing (NADH 1) from four regions; *salmo trutta fario* -Jaj

Roud, *salmo trutta fario* -Siahkal Roud, *salmo trutta fario* -Cheshme kileh and *salmo trutta fario* Ghasem Abad by DNAMAN Demo 8.0 program. The result showed high homology between sequences. Table 2. The nucleotide composition of the coding strand of mitochondrial genome compared between *salmo trutta caspius*, *salmo trutta*, *salmo salar* and *salmo trutta fario* illustrated a bias against the use of four DNA nucleotides (A-T-C-G) with the range of 13.6 to 36.9.almost the range of nucleotides were same between salmonid species used this study.

Table 1 : The number of meristical variables in *salmo trutta fario* population from four regions of rivers in Iran (Cheshme kileh(A), Ghasem Abad(B), Siahkal Roud(C) and Jaj Roud(D)

Row	Counting factors	A				B				C				D			
		Min	Max	Mean	S.D	Min	Max	Mean	S.D	Min	Max	Mean	S.D	Min	Max	Mean	S.D
1	Scales on lateral line	110	134	120	2.83	110	133	119	2.80	109	132	118	2.08	110	133	120	2.07
2	Scales above the lateral line	25	33	28.07	1.42	24	32	28.2	1.40	24	32	28.4	1.41	25	32	28.07	1.40
3	Scales below the lateral line	18	27	27.72	1.60	19	28	22.80	1.62	19	28	22.76	1.61	18	28	22.72	1.60
4	Scales between the lateral line and fin fat	15	21	17.98	1.21	15	21	17.99	1.22	15	22	17.99	1.21	15	21	17.98	1.21
5	Number of spines in Dorsal fin	3	5	4.23	0.44	3	6	4.54	0.45	3	6	4.55	0.44	3	6	4.23	0.43
6	Number of soft rays in Dorsal fin	8	10	9.51	0.62	9	11	9.55	0.65	9	11	9.54	0.61	8	11	9.51	0.61
7	Number of spines in Anal fin	3	5	3.12	0.51	3	6	3.11	0.52	3	6	3.10	0.51	3	6	3.12	0.51
8	Number of soft rays in Anal fin	7	10	9.10	0.66	6	9	8.10	0.64	6	9	8.11	0.63	7	9	9.10	0.61
9	Number of spines in ventral fin	1	1	1.00	0	1	1	1.00	0	1	1	1.00	0	1	1	1.00	0
10	Number of soft rays in ventral fin	7	9	7.71	0.42	6	8	7.12	0.44	6	8	7.12	0.40	7	8	7.71	0.40
11	Number of spines in pectoral fin	1	1	1.00	0	1	1	1.00	0	1	1	1.00	0	1	1	1.00	0
12	Number of soft rays in pectoral fin	11	12	11.31	0.45	11	12	11.21	0.44	10	12	11.23	0.42	11	12	11.31	0.42
13	soft rays of caudal fin	16	17	16.12	0.19	16	18	16.45	0.20	17	18	16.49	0.21	16	18	16.12	0.21
14	Gill rakers	16	22	17.98	1.25	17	23	18.1	1.27	17	24	18.2	1.26	16	23	17.98	1.26
15	Gill filaments	10	13	10.65	0.49	11	14	11.65	0.51	11	14	11.68	0.52	10	14	10.65	0.52
16	Number of trunk vertebrat	59	62	59.05	0.99	58	63	59.99	099	58	65	59.99	0.99	59	63	59.05	0.99
17	Pyloric coeca	32	50	39.10	3.29	33	51	39.99	3.34	34	52	40.11	3.38	32	51	39.10	3.38

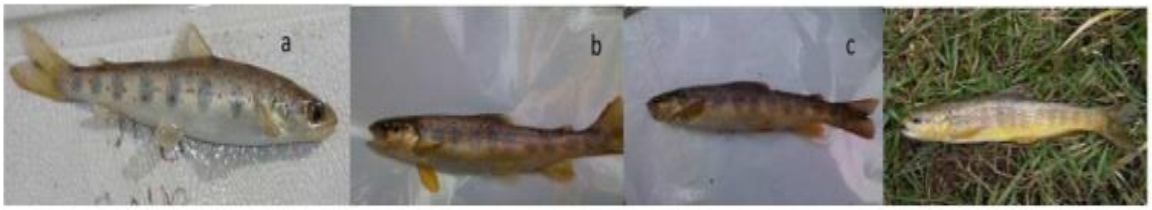

Fig. 1. The results of mean value of morphometrically analysis of *salmo trutta fario* between four regions of Cheshme kileh(a), Ghasem Abad(b), Siahkal Roud(C) and Jaj Roud(D).

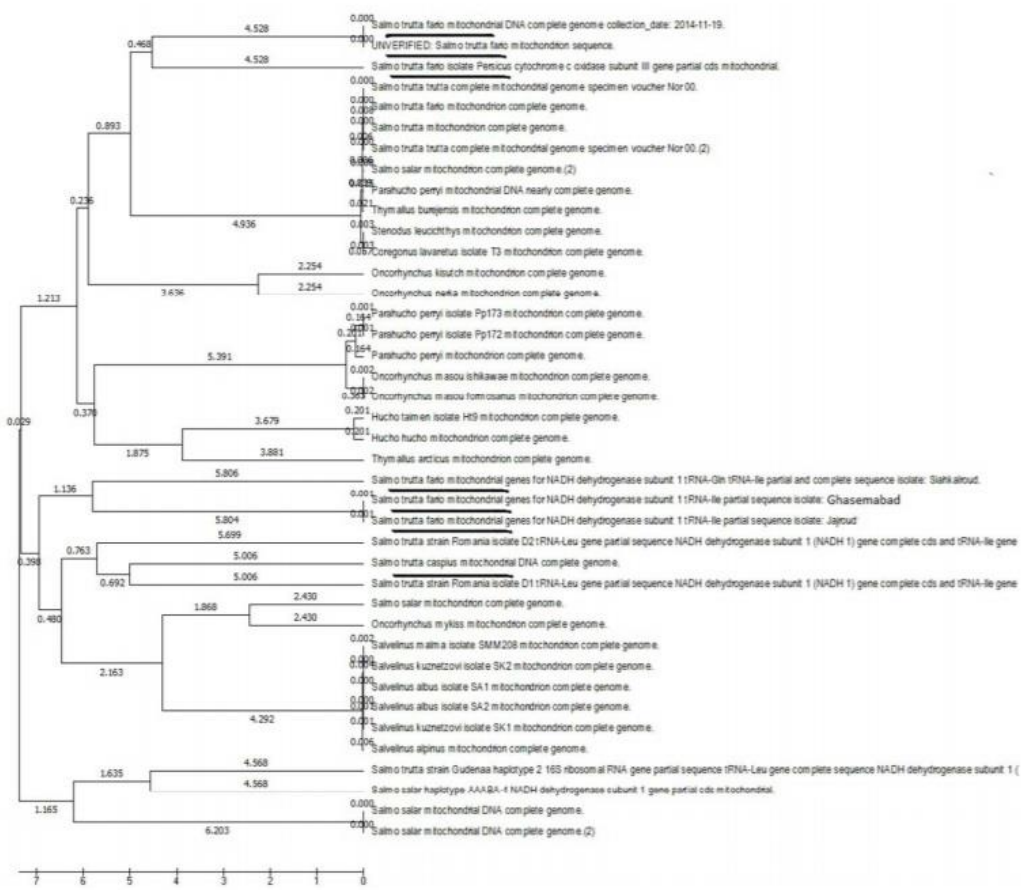

Fig. 2. The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 103.769 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 40 nucleotide sequences. All positions containing gaps and missing data were eliminated.

Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution

	A	T	C	G
A	-	*4.55*	*4.85*	**18.73**
T	*4.82*	-	**8.4**	*2.94*
C	*4.82*	**7.88**	-	*2.94*
G	**30.67**	*4.55*	*4.85*	-

Fig. 3. Maximum Composite Likelihood of the pattern of nucleotide substitution from one base (row) to another base (column). Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. The nucleotide frequencies are 29.76 %(A), 26.34% (T/U), 24.24% (C), and 19.66% (G). All positions containing gaps and missing data were 705 positions in the final dataset showed by MEGA 7.

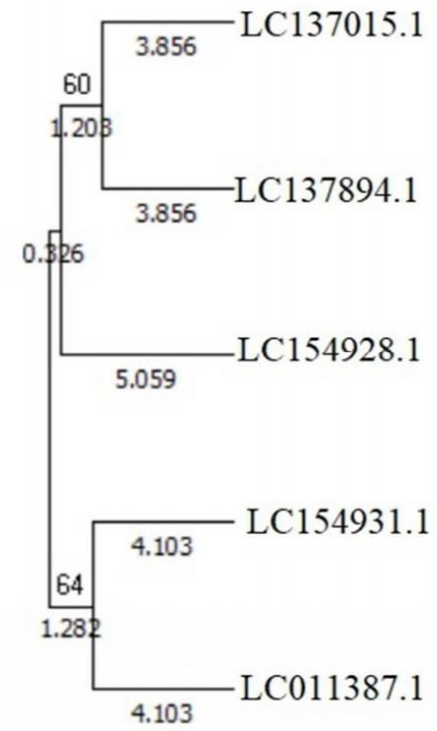

Fig. 4. Evolutionary analysis of *salmo trutta fario* isolate Persicus used this study was inferred using the UPGMA method. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. According to the length of branch, *salmo trutta fario* -Jaj Roud 2(LC154931.1), *salmo trutta fario* -Siahkal Roud (LC1549281), *salmo trutta fario* –Cheshme kileh (LC137015), and *salmo trutta fario* -Ghasem Abad (LC137894), variety were different respectively.

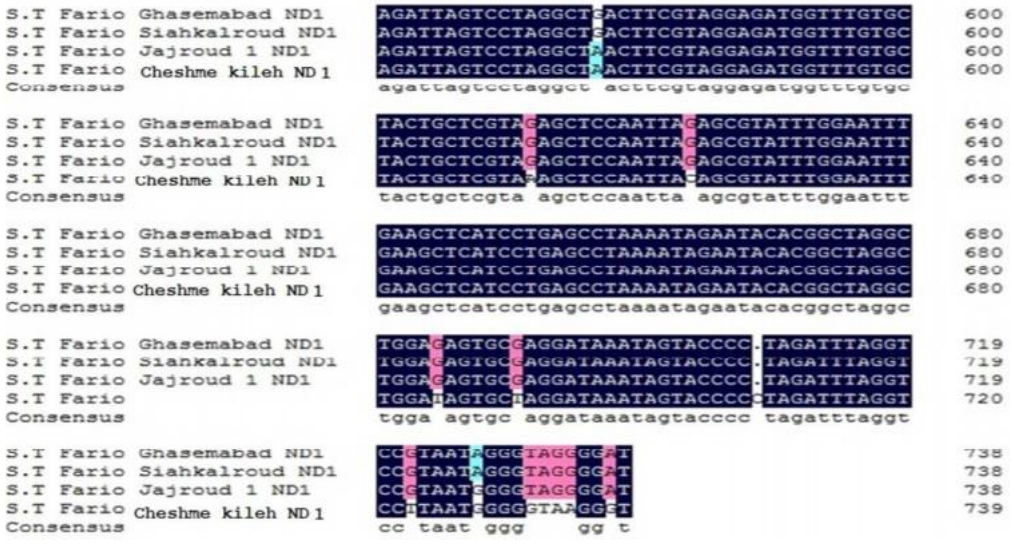

Fig. 5. Accession numbers of five sequences of partial mitochondrial sequencing (NADH 1). According to the length of the branch, *salmo trutta fario* -Jaj Roud, *salmo trutta fario* -Siahkal Roud, *salmo trutta fario* Cheshme kileh and *salmo trutta fario* -Ghasem Abad. The results showed high homology between sequences under investigation.The results conducted by DNAMAN Demo 8.0.

Table 2. The nucleotide composition of the coding strand of mitochondrial genome compared between *salmo trutta fario* and *salmo trutta caspius*, *salmo trutta*, *salmo salar* illustrated a bias against the use of four DNA nucleotides (A- T- C- G) with the range of 14.1 to 36.9 .

	Salmo trutta fario				*Salmo trutta caspius*				*Salmo trutta*				*Salmo salar*			
Nucleotide	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G
D-loop	31.3	22.1	31.3	14.0	31.6	22.3	31.2	14.1	31.4	22.4	31.7	14.4	31.7	22.2	31.5	14.7
12SrRNA	19.6	27.4	29.1	22.5	19.2	27.8	29.7	22.6	19.5	27.3	29.5	22.3	19.8	27.9	30.5	22.5
16SrRNA	19.8	25.9	32.5	22.5	19.8	25.6	32.5	22.5	19.4	25.4	32.3	22.4	19.5	25.8	32.6	22.3
ND1	27.0	32.6	27.0	14.5	26.8	31.2	26.9	15.4	26.8	32.4	26.9	14.3	27.5	31.6	25.9	15.3
ND2	25.7	35.1	26.1	13.7	25.1	34.2	26.9	13.9	25.6	35.3	26.3	13.6	26.5	33.7	28.4	11
ND3	27.4	34.3	22.7	15.3	26.9	33.0	24.1	16.0	27.4	34.3	22.7	15.3	27.5	34.6	23.4	14.8
ND4	27.3	30.4	27.7	13.4	27.7	30.6	27.2	14.0	27.4	30.2	27.6	13.3	28.4	30.5	27.7	14.8
ND4L	24.5	34.2	24.9	16.4	24.2	34.3	24.9	16.5	24.6	34.3	24.8	16.3	26.6	32.4	24.7	16.8
ND5	27.6	30.5	28.1	13.8	27.5	30.6	28.4	13.6	27.5	30.4	28.3	13.7	27.7	30.6	29.8	12.7
ND6	14.9	34.4	37.2	13.6	14.5	35.0	36.9	13.6	14.8	34.5	37.3	13.7	15.6	36.7	38.6	12.9
COX1	19.5	27.3	29.5	22.3	28.1	28.1	27.8	16.1	19.5	27.3	29.5	22.3	30.5	27.5	24.8	17.4
COX2	29.1	28.6	24.8	13.2	29.1	31.5	24.0	15.3	29.5	28.7	24.6	13.3	28.5	27.8	27.7	16.5
COX3	29.5	29.6	24.2	17.0	29.5	29.1	24.6	16.3	29.4	29.5	24.3	16.9	28.8	29.6	26.3	15.7
ATPase8	25.4	32.4	29.8	12.3	25.0	32.7	29.8	12.5	25.4	32.4	29.8	12.3	26.8	31.7	29.8	12.6
ATPase6	28.2	34.3	25.6	12.9	27.5	34.5	25.1	12.5	28.4	34.4	25.5	12.8	28.4	33.5	26.8	11.9
Cytb	29.4	31.2	24.8	15.2	29.2	31.5	24.5	15.7	29.3	31.3	24.7	15.4	29.6	30.8	24.0	15.9

Fig. 6. Mapping of four regions of sampling *salmo trutta fario* isolate Persicus in this study. The distance between Siahkal Roud in Gillan province to Jaj Roud in Tehran province is more than 244 kilometers.

DISCUSSION AND CONCLUSION

Status of Living *Salmo trutta fario* in the World:

Salmo trutta fario (Linnaeus, 1758) is native in some rivers in the Mediterranean, the Black Sea (at least in upper Danube drainage), and the Caspian Sea (at least in upper Volga drainage (Yousefian, 2011). Furthermore, *Salmo trutta fario* is one of the salmonid species in the IUCN Red List of Threatened Species (Coad, 1980), because pollution of rivers and aquifers, loss of habitats and natural spawning areas, decreased precipitation (Johnson *et al.,* 2008) collection of sand, gravel and changes river glens (Ciftci and Okumus, 2002), obstacles to migration from the sea to rivers when spawning, such as bridges and dams, and thus, blockage of migration routes (Dionne) *et al.*, 2008), and the pollution of rivers causes reduction and death of

salmonid population. Hence, it is important to study the phylogenetics of salmonids (e.g. *salmo trutta fario).*

Sequence analysis of complete mitochondrial genomics in *Salmo trutta fario* was analysed with the discovery of genes controlling the mitochondrial genomes. Review on this organelle is one of the important new perspectives in genetic and phylogenetic studies (Magoulas and Zouros, 1993). *Salmo trutta fario* usually lives in freshwater rivers, lakes and feeds in autumn to spawn upstream of the rivers in fall. These species are very sensitive to climate change. Hence, salmonid species living in the sea are more genetically diverse than salmonid species lives in freshwaters such as in rivers and lakes (Jamshidi and Kalbasi, 2011). Thus, in *Salmo trutta fario*, diversity is less than the other salmonids living in the sea, and thus, gene flow and population size should be smaller in value (Rossy *et al.,* 2004). A nucleotide sequence substitute occurs in the interval of species, which is entitled transition and transversion. Transition occurs when a purine base (A,G) converts to other bases of Purine or a Pyrimidine bases (C to T) or converts to other Pyrimidine bases. Furthermore, transversion occurs when a Purine converts to a Pyrimidine base and vice versa. Currently, these two factors are considered as indicators of molecular diversity in salmonid species (Tamura *et al.*, 2004). In this study, the number of transitions and transversions of salmonid species between *salmo trutta fario* and other salmonids studied in this research was high (Fig. 3). Therefore, it was concluded that the situation of transition and transversion between *salmo trutta fario* (the molecular diversity) is different. On the other hand, species traits, similarities and evolutionary analyses were studied by Maximum Parsimony between *salmo trutta fario* isolate Persicus (LC137015) and other salmonids. Results showed a variation between salmonid species. *Salmo trutta fario* and *Oncorhynchus mykiss* (LC050735), *Stenodus leucichthys* (KX151784) were in one branch, while other Iranian salmonids (*Salmo trutta caspius*) resided in another branch (Fig. 2). *Salmo trutta trutta* (AM910409) and *Salmo salar* (U12143) are in one branch, and *Salmo trutta fario* (KT633607) and *Salmo salar* (JQ390056) are in another branch. Hence, according to Fig. 2, ancestral states for salmonids species were different; however, the results show a high nucleotide homology between them. According to Rezaei *et al.* (2017) twenty sample of *Salmo trutta caspius* from three local regions (Safa Roud, Nav Roud and Cheshmeh Kileh) were examined and compared with other salmonid species such as *Salmo trutta, Salmo salar* and *Oncorhynchus mykiss*. They found high homology between the samples. Furthermore, Fig. 4 shows that no significant variation was found between the evolutionary relationship between taxa in the intergroup of *Salmo trutta fario*. Figure 5 also shows high homology between three *Salmo trutta fario* habitats in Iran in three major rivers of Jaj Roud, Siahkal Roud and Ghasem Abad connected to south of Caspian Sea. According to Rezaei *et al.* (2017) phylogenetic analysis of *Salmo trutta caspius* species was examined in three major rivers (Nav Roud, Cheshmeh Kileh and Safa Roud) connected to south of Caspian Sea. They showed high homology between the intergroup of *Salmo trutta caspius* species while populations were collected from geographical chains stretching from the south (Tonekabon city) to the south-west of rivers connected to the Caspian Sea (Talesh city). The distance between Siahkal Roud in Gillan province and Jaj Roud in Tehran province (the furthermost region in this study) was244.4 kilometers (Fig. 6). However, the distance from Siahkal Roud to Jaj Roud is significant; nonetheless, distance is not a major reason for diversity in salmonids species. Eliot (1994) studied the phylogenetic status of *Salmo trutta caspius* in several regions including populations in Iranian inland basins and the Persian Gulf basin (Turkey), which shows good similarity with North African populations that were

previously unknown. Segherloo et al. (2012) used the complete mtDNA control region to compare the Iranian *Salmo trutta* populations of seven haplotypes including HKa (Karaj River), HH1, HH2 (Haraz River), HBa (Babolrud River), HCo (the most frequent haplotype in all the studied rivers except for Karaj River), HM1 and HM2 (Mardagh River) which showed genetic diversity between the studied subjects.

In this research, *Salmo trutta fario* isolated Persicus was collected from four regions (Jaj Roud, Cheshme Kileh, Ghasem Abad and Siahkal Roud) for the purpose of phylogenetic analysis between them using mitochondrial genomics. *Salmo trutta fario* are native in Iran and live in major rivers including Lar, Plor, Karaj, Shirud, Tajan, Haraz, Tonekabon, Havigh, Shafarud, Chesli, Sefidrud and many rivers of Mazandaran and Gillan provinces (Salavatian *et al.,* 2011; [Abdoli, 2000; ,Kiabi, 1999; Abdoli, 2008; Kazancheev, 1981). Salavatian *et al.* (2011) also reported that almost 99% of *Salmo trutta fario* living in Lar Lake were maintained and appropriate measures have been taken to preserve the species. On the other hand, other researchers have conducted phylogenetic studies in the major rivers of Azerbaijan (Quliyev, 2006) and in Lithuania (Skrupskelis, 2006).Moreover, *Salmo trutta fario* is one of the top four threatened species of freshwater fishes, and this species is endangered in Iran because of overfishing, destruction of their habitat and spawning ground degradation (Nezami *et al.*, 2000). Table 1 shows the mean value of phenotypic characterization based on 17 meristic traits of *Salmo trutta fario* from four regions (Cheshme kileh, Ghasem Abad, Siahkal Roud and Jaj Roud). According to our results, we observed the same mean value of phenotypic meristic in the four regions; thus, the proposed region does not show any significant variation between *Salmo trutta fario* populations. Comparisons of the meristical data between the *Salmo trutta fario* population in Lar Lake have also been conducted by Salavatian *et al.* (2011) in which they found the same range of meristical data (scales number, etc.). Moreover, the same results were found on the *Salmo trutta fario* population in major rivers of the countries of Azerbaijan and Lithuania by Quliyev (2006), Skrupskelis (2006) . Complete mitochondrial genome is a suitable method for phylogenetic studies used in this study. A similar method was also used by Tiano (2005) regarding Brown trout that found two haplotypes within Gilchrist strain and Wild Rose strain. Our results were analyzed using different options of MEGA software version 7 including Maximum Parsimony analysis of taxa and Maximum Composite Likelihood Estimate (figures 2 and 3) between *Salmo trutta fario* populations and also within the group of *Salmo trutta fario* aligned by DNAMAN software showed high homology between salmonid species. A phylogenetic tree containing *Salmo trutta fario* and other salmonids like *Salmo salar, Onchorhynchus mykiss* and *Salmo trutta caspius*, etc., was constructed to further investigate their genetic relatedness (see figures 2, 3, 4 and 5). This tree grouped all salmonids in accordance with their conventional phylogeny; the genus Salmonid formed one branch (*Salmo trutta fario, Stenodus leucichthys* and *Onchorhynchus mykiss* and *Salmo salar*) and two genus of *Salmo* (*Salmo salar*; different accession numbers) and *Oncorhynchus* (different accession numbers) formed another branch (Fig. 2).

Finally, low genetic variability was found between the populations of salmonids, *Salmo trutta fario, Salmo trutta trutta, Salmo trutta caspius* and *Oncorhyncus mykiss*. In the current study, may be related to life history characteristics of the particular populations that are connected geographically. Similar studies have been carried out by Bernatchez (2001), Wills (2006), Tiano (2005) and Apostolidis *et al.*, (1997) on *brown trout* populations by partial mitochondrial genomes such as ND1 and ND5/6 genes. They proposed that *Brown trout* populations had limited spawning and nursery conditions in their environment compared to other populations of salmons. A typical

recombinant resulting in low effective population sizes or repeated bottlenecks could lead to a loss of genetic variability. However, isolation combined with a population bottleneck and genetic drift could result in significant genetic differentiation among *brown trout* populations. Furthermore, phylogenetic trees would be expected to display conservation as a protein-coding region by mitochondrial genes group revealed by Nilsson *et al.* (2002) and Cornell and Ward (2000). Additionally, relatively constant rates of mutation were observed among different salmonid species by these genes (Doiron et al. 2002). The current study used full and partial mitochondrial genomes between and within salmonid species; however, it is suggested to use nuclear genomics as means for phylogenetic studies.

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