Research Article [Araştırma Makalesi]

Heteroplasmy and length variation in the tRNApro-Dloop regions of three sturgeon species (*A. stellatus, A. gueldenstaedtii and H. huso*) from the Turkish coast of the Black Sea

[Karadeniz'in Türkiye kıyılarından örneklenen üç mersin balığı türü (*A. stellatus, A. gueldenstaedtii ve H. huso*)'nün tRNApro-Dloop bölgelerinde heteroplazmi ve boy varyasyonu]

Yılmaz Çiftci[1],
Oğuzhan Eroğlu[2],
Şirin Firidin[2]

[1]Ordu University, Faculty of Marine Sciences, Department of Fisheries Technology Engineering Ordu, Turkey
[2]Ministry of Food, Agriculture and Livestock, Central Fisheries Research Institute, Department of Genetics, Trabzon, Turkey

ABSTRACT

Objective: To determine tandem repeat polymorphism and heteroplasmy in three sturgeon species (*A. stellatus, A. gueldenstaedtii and H. huso*) from the Turkish coast of Black Sea.

Methods: tRNApro and D-loop segment of mtDNA from three sturgeon species were amplified via PCR and sequenced. For each species, homoplasmic individuals with different product lengths, repeat motifs and regions were determined and the repeat numbers and frequencies were calculated. The variation in mtDNA size present in the overall sample of sturgeon from Turkish waters was apportioned into hierarchical components. Also, the statistical approach described by other researchers was used for the calculation of inter-species and intra-species genetic variation.

Results: The results showed that all three species reveal 2-6 copies of different mtDNA length variants attributable to varying copy numbers of an 82-84bp repeat sequences. A total of 9.9% of the sturgeons were heteroplasmic, bearing three to five repeat variants. The highest number of observed repeat units rate was 45.8% in 3 repeats morph in *A. gueldenstaedtii*. The mean genetic diversity within individuals (Kb) was higher in *A. gueldenstaedtii* and *A. stellatus* than in *H. huso* (0.625, 0.620, and 0.500, respectively).

Conclusion: The repeat region, responsible for length variations and heteroplasmy, is located near the end of the D-loop and control region separated by only a few nucleotides from the tRNApro gene.

Key Words: sturgeon, Turkey, Black Sea, tRNApro-Dloop, heteroplasmy, length variation

Conflict of Interest: There is no conflict of interest in respect of this manuscript.

Yazışma Adresi
[Correspondence Address]

Yılmaz Çiftci

Ordu Üniversitesi, Fatsa Deniz Bilimleri Fakültesi, Fatsa, 52400, Ordu, Türkiye
Tel. +90 452 423 50 53
E-mail. yciftci@gmail.com; yciftci@odu.edu.tr

ÖZET

Amaç: Karadeniz'in Türkiye sahillerinden örneklenen 3 Mersin balığı türünde (*A. stellatus, A. gueldenstaedtii ve H. huso*) heteroplazmi ve sıralı tekrar polimorfizminin belirlenmesi.

Yöntemler: Üç mersin balığı türünde mtDNA'nın tRNApro ve D-loop segmenti PCR yardımı ile yükseltgenmiş ve nükleotit dizilimleri belirlenmiştir. Her bir tür için, farklı ürün boyuna, tekrar motiflerine ve bölgelerine sahip homoplazmik bireyler tespit edilmiş ve tekrar sayıları ve frekansları hesaplanmıştır. Türkiye sularından mersin balığı örneklerinin hepsinde bulunan mtDNA'daki varyasyon hiyerarşik bileşenlerine paylaştırılmıştır. Ayrıca, tür içi ve türler arası genetik varyasyonun hesaplanmasında diğer araştırmacıların tanımladığı istatistiksel yaklaşımlar kullanılmıştır.

Bulgular: Çalışma bulgularına göre, üç mersin balığı türü 82-84 baz çifti içeren tekrar dizilerinin değişen kopya sayılarına atfedilebilecek 2-6 tekrarlı, farklı mtDNA boy türevlerini ortaya çıkarır. Mersin balıklarının %9.9'u 3-5 tekrar türevlerine sahip heteroplazmiktir. En fazla gözlenen tekrar birimi *A. gueldenstaedtii* örneklerinde %45,8 oranı ile 3 tekrarlı morf olmuştur. Tür içi ortalama genetik farklılık (Kb) *A. gueldenstaedtii* ve *A. stellatus*'da *H. huso*'dan daha yüksektir (0,625, 0,620, ve 0,500, veriliş sırasıyla).

Sonuç: Boy varyasyonu ve heteroplazmiden sorumlu olan tekrar bölgesi, D-loop bölgesinin sonuna yakın konumdadır ve kontrol bölgesi tRNApro geninden birkaç nükleotid ile ayrılmaktadır.

Anahtar Kelimeler: Mersin balığı, Türkiye, Karadeniz, tRNApro-Dloop, heteroplazmi, boy varyasyonu

Çıkar çatışması: Yazarlar arasında çıkar çatışması yoktur.

Introduction

The mitochondrial genome (mtDNA) is a relatively small, circular and double-stranded DNA molecule that consist of 16.000-17.000 bp in fishes [1], encodes 13 polypeptides, as well as 22 tRNAs and two rRNAs that contribute to mitochondrial protein synthesis. Most individuals of most vertebrate species possess a single form of mtDNA that is inherited from the female parent. Nevertheless some individuals and some species exhibit more than one form of mtDNA [2]. This condition, known as heteroplasmy, has been reported in several cartilaginous and bony fish [3-8]. There are large random shifts in heteroplasmy level between female parent and offspring. The distribution in heteroplasmy levels across a group of offspring is an important step in order to understand the combination of haploidy and maternal inheritance of mtDNA in species. Furthermore, the existence of heteroplasmic cytoplasms provides an opportunity to examine the action of genetic drift, mutation and selection [9].

Intra-species variations in mtDNA length in fish can be as large as inter-species differences [10]. In higher vertebrates, inheritance is only maternal (heteroplasmy may be observed in certain species), and it is functionally haploid due to the apparent absence of recombination [11]. It is observed that the paternal mitochondria are broken down during fertilization. In addition to this, paternal leakage can occur at a low rate such as once in 1000 molecules. Concerning heteroplasmy, there are increasingly more reports regarding the existence of more than one type of mtDNA in individuals. Heteroplasmy does occur, albeit rarely, in many fundamental groups of organisms, which includes certain species of fish. Sturgeon [3,4,12], codfish [9] and mussel species [13] can be provided as examples.

Among all mitochondrial genes, the control region has the high rate of change. The rate of evolution of the control region is 2-5 times more than the rate of evolution of genes that encode mitochondrial proteins [14]. Synthesis of the D-loop strand is initiated near the conserved sequence blocks (CSBs), runs toward the tRNA pro-gene and terminates beyond the terminal associated sequence (TAS) [15]. Due to the tandem duplication of control regions, their length varies between 200-4100 bp in length. For this reason, they are the main subject of studies on the length variability of mitochondrial genome in vertebrates. In addition, the population has a high frequency of fragment mutations. When heteroplasmy is observed in fish, tandem duplication and repetitions of different lengths are also observed. As mutations accumulate rapidly in the control region, it is a preferred molecule for studies on the phylogenetic relation between species with very similar control regions, and for studies one population levels.

The Acipenseriform group (sturgeon and paddlefish) are the subject of our study and represent a species that is currently endangered with extinction. They are an ancient group that has persisted since the Jurassic times until our era, with 27 currently existing species [16]. It has been reported that 6 species exist in the Black Sea [17].

Recent molecular studies indicate that the origins of this order stretches to 200 million years ago, that the Acipenseriforms originate from the Tethys basin, and that the main center for their differentiation was the Black Sea [18]. In this context, Turkey occupies a very important location. If Chondrosteidae and Peipiaosteidae are sibling groups, the Acipenseriform order has its origins in Europe in the Early Jurassic era, with species diversification occurring in Asia during the Late Jurassic/Early Cretaceous in Asia and later in North America [19]. With a few exceptions, all remaining species other than *Acipenser fulvescens*, *A. brevirostrum*, *A. baerii* are local species of the Black Sea-Caspian region. Berg [20] suggested that these anadrome species originate from the "grand children" of the Tethys Sea, which are the Caspian Sea, the Aral Sea, the Black Sea and the Azov Sea and other regions that formed later.

In this study, we aimed to determine tandem repeat polymorphism and heteroplasmy in three sturgeon species (*A. stellatus*, *A. gueldenstaedtii* and *H. huso*) from the Turkish coast of Black Sea. The sequence analysis results of the tRNA[pro]-Dloop gene regions were used as genetic data. Although there are other studies performed by sampling the Black Sea, these studies do not include samples from the coasts of Turkey. In the previous studies conducted on sturgeon species from the Black Sea coast of Turkey, several sub-species were identified based on morphological and meristic characteristics. As this study is the first molecular study on sturgeon species, it will, to a certain extent, help close the gap in knowledge on this topic, and will also form a basis of future studies.

Materials and Methods

Sampling and DNA extraction

A total of 12 Stellate sturgeon (*A. stellatus*), 24 Russian sturgeon (*A. gueldenstaedtii*) and 45 Beluga (*H. huso*) were used. All samples used in this study were collected from 8 different localities (Rize, Trabzon, Giresun, Ordu, Samsun, Sakarya, Istanbul and hatchery originating samples from Russia) on the Turkish coast of the Black Sea (Table 1).

Around the 1-2 cm^2 caudal fin was removed in field without harming the fish itself and stored in 98% ethanol. All samples were brought to the laboratory to be stored at -20°C in a deep freeze until DNA extraction.

Total genomic DNA was extracted using a standart proteinase K, Phenol-Chloroform method with ethanol precipitation, or by the DNA extraction kit (QIAGEN,

Table 1. Sampling location of sturgeon species used in this study

Sampling locations	Species			TOTAL
	A. stellatus	*A. gueldenstaedtii*	*H. huso*	
Rize	-	-	2	2
Trabzon	4	1	11	**16**
Giresun	5	5	18	**28**
Ordu	1	-	5	**6**
Samsun	2	6	8	16
Sakarya	-	2	-	**2**
İstanbul	-	-	1	**1**
Hatchery orginated samples from Russia	-	10	-	**10**
TOTAL	12	24	45	81

DNeasy® Tissue kit). The DNA concentration and quality was assessed spectrophotometrically at 260/280 nm.

PCR amplification and sequencing

tRNA^{pro} and D-loop segement of mtDNA from three sturgeon samples were amplified via Thermal Cycler using one set of primers (Hetero I) 5'-ACCCTTAACTCCCAAAG-3' and (HeteroII) 5'-CATTTRATGGTAGATGAAAC-3 developed by Ludwig et al. [4]. Amplifications were carried out in a 50µl final volume containing 6µl of each primer (10pM), 10µl DNA template (100ng/µl), 3µl ddH2O and 25 µl 2X PCR Master Mix (Promega), which is a premixed and ready-to-use solution containing Taq DNA Polymerase (50unit/ml), dNTPs (400 µM dATP, 400µM dGTP, 400µM dCTP, 400 µM dTTP), MgCl$_2$ (3mM) and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. The PCR reactions for tRNA^{pro}-D-loop segment were performed with a PTC-200 gradient thermal cycler (MJ Research, Waltham, Massachusetts, USA), with an initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30s and extension at 72°C for 60s and final extension for 4 min at 72°C. As a result of PCR amplification, 5µl-product was run in 1% agarose gel together with a 1xTBE buffer system and was visualized and controlled by a UV illuminator by being stained with ethidium bromide.

Purification of successfully amplified PCR product obtained from samples of different product size was performed by Wizard® SV Gel and PCR Clean-Up System (Promega) kit according to the manufacturer's instructions. Following purification, by diluting pure DNA with 50 µl and running 1 µl on agarose gel using a procedure similar as before, the effectiveness of the procedure and the concentration of the obtained pure DNA fragments were evaluated. It was then sent to a commercial laboratory with bi-directional PCR primers and sequenced.

Data Analyses

Nucleotide sequences of the 250-600 bp tRNA^{pro} and D-loop segement of mtDNA were determined for 15 sturgeon individuals. The forward and reverse sequences were aligned to form a composite fragment of mtDNA for each fish using BioEdit v. 7.0.4.1 [21] and MEGA 3.0 [22]. For comparative analysis, sequences of mtDNA regions were combined with the sequences from the current study and used to infer the genetic relationship of sturgeons in the certain Black sea localities in Turkey.

Length variation and heteroplasmy for each species was demonstrated by running the tRNA^{pro}-Dloop gene PCR product on agarose gel and comparing the resulting images. For each species, homoplasmic individuals with different product lengths were identified for sequencing. Following the retrieving of sequence data, repeat motifs and regions were determined and the repeat numbers and frequencies were calculated for each species. Length variation and heteroplasmy was identified in the gel images of 81 sturgeons belonging to three species.

The variation in mtDNA size present in the overall sample of sturgeon from Turkish waters was apportioned into hierarchical components as described in Rand and Harrison [23]. The statistical approach of Birky *et al*. [24] was used for the calculation of inter-species and intra-species genetic variation. For each fish, the probability of sampling different genes was calculated with the aid of the equation as follows: $Kb = (1-\Sigma\ x_i^2)$, where x_i is the length variation rate for each sample (fish). The Kb value was taken as "0" in homoplasmic samples. For each population subset, an average of the Kb value was calculated such that both homoplasmic and heteroplasmic individuals would be included. Of these values calculated for all individuals, the gene probability (Kc) within the species could display variation. This value was determined as follows:

$Kc = (1-\Sigma x_i^2)$, where x_i is the average frequency of the ith length variant within the population. In this way, it was ensured that Kc includes heteroplasmy [24]. The G_{lp} equation, on the other hand, was used to demonstrate

the rate of intra-species gene variation. It was calculated with the aid of the equation as follows: $G_{IP} = (Kc-Kb)/Kc$

Results

Length variation and heteroplasmy

MtDNA control region length variation and heteroplasmy was assessed in 81 samples from *H. huso, A. stellatus* and *A. gueldenstaedtii* (Table 2 and Figure 1). The length variation in PCR products was due to the differing number of repeating units. When length variation was evaluated in these samples, 45.8% of *A. gueldenstaedtii* samples had 3 repeating sequences, making them the most frequently encountered morph. While the lowest repeat number was determined as 2, the highest repeat number was 6. For all species, the distribution rate of the average length variation was respectively 3.7% for 2 repeats, 37% for 3 repeats, 33.3% for 3 repeats, 8.6% for 5 repeats, and 7.4% for 6 repeats. The 3 repeats were the most frequently encountered. Among the evaluated species, 1 or 7 repeat sequences were not observed.

For each fish, different mitochondrial genomes were identified by means of length differences in the PCR products. Samples with only a single mtDNA D-loop were considered as homoplasmic, while samples with more than one mtDNA D-loop were considered as heteroplasmic (Figure 1). Among the evaluated species, heteroplasmy was observed at a rate of 9.9%. Accordingly, the rate of homoplasmy was calculated as 90.1% (Table 3). In the *H. huso* species, heteroplasmy was observed in only one individual. With a rate of heteroplasmy of 2.2%, *H. huso* was determined as having the lowest heteroplasmy among all the species (Table 3).

By using Birky *et al*.'s [24] statistical approach, inter-species and intra-species genetic variation for each fish was calculated by using the probability of the sampled gene size. The Kb value was taken as "0" in homoplasmic samples. While the largest Kb value of 0.0625 was observed in *A. gueldenstaedtii*, Kb values were calculated as 0.620 for *A. stellatus* and 0.500 for *H. huso*. Contrary to the Kb values, the G_{IP} values were lowest in *A. gueldenstaedtii* with a value of 0.063. Kb was determined as 0.203 in *A. stellatus*, and the highest value was as 0.285 in *H. huso* (Table 3).
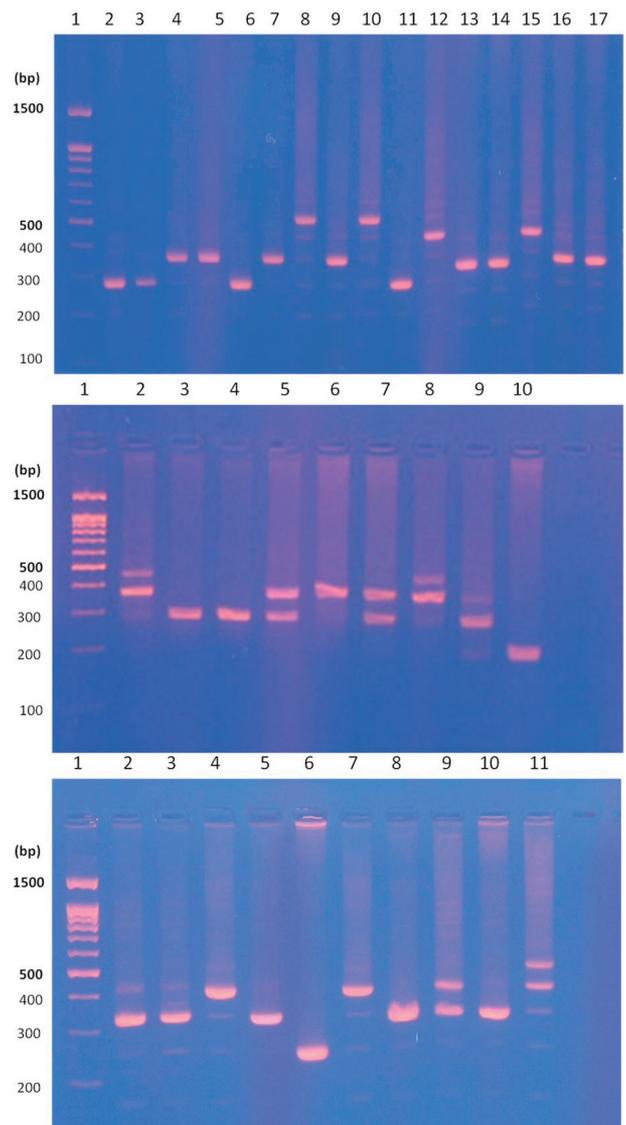
Figure 1. Electrophoretic patterns of length variation and heteroplasmy for mtDNA, tRNA^{pro} - Dloop segment of sturgeon species (*H. huso* (upper), *A. stellatus* (middle) *and A. gueldenstaedtii* (bottom)).

Table 2. Frequency and distribution of mtDNA size classes in the sturgeon species analyzed in this study

Species		Size classes						
	n	f(2)	f(3)	f(4)	f(5)	f(6)	f(3-4)	f(4-5)
H.huso	45	0	0.333	0.400	0.111	0.133	0.022	0
A.stellatus	12	0.083	0.333	0.083	0.083	0.000	0.167	0.250
A.gueldenstaedtii	24	0.083	0.458	0.333	0.042	0.000	0.042	0.042
Total	81	0.037	0.370	0.333	0.086	0.074	0.049	0.049

Note: n = sample size, f(3-4) = frequency of the **3-4** size class

Table 3. Frequencies of homoplasmic and heteroplasmic sturgeons and diversity indices in pooled samples

Species	Homoplasmic	Heteroplasmic	Diversity indices		
			K_b	K_c	G_{IP}
H.huso	0.978	0.022	0.500	0.699	0.285
A.stellatus	0.583	0.417	0.620	0.778	0.203
A.gueldenstaedtii	0.917	0.083	0.625	0.667	0.063
Totals	0.901	0.099	0.616	0.733	0.160

Kc = diversity measure for the combined sample of size classes from the level of grouping, Kb = mean diversity measure for all individuals in the level of grouping, $G_{IP} = (Kc - Kb)/Kc$ where G_{IP} = the proportion of gene diversity within a species attributable to between fish diversity.

Sequence analysis

From a total of 19 samples belonging to 3 different species, which were the *A. gueldenstaedtii* (8), *A. stellatus* (4) and the *H. huso* (7), the partial sequence of the tRNA[pro]-D-loop region was taken forwards and reverse, in both directions. By overlapping the sequences in both directions, a common sequence of a total of 250-600 bp in length was obtained. BLAST (The Basic Local Alignment Search Tool) analysis was performed on the resulting sequence obtained in the following stage in order to identify regions with local similarity between the sequences, to make comparisons with sequences in international databases, and also to determine the species to which the studied gene belongs to.

By determining the tRNA[pro] gene region sequence within the tRNA[pro]-D-loop sequence, the initial TAS (Termination Associated Sequence) region was determined, and the control region and tRNA[pro] were distinguished from one another. It was observed that the repeat region near the end of the D-loop was responsible for the length variation and heteroplasmy. The control region is separated from the tRNA[pro] by only a few nucleotides. These repeats always have sequences (TAS) related to the termination of the D-loop strand. The TAS-like block can have a determining role within the tRNA[pro]. Certain repetitive base sequences were identified by considering the central repeats of the repeat regions. Accordingly, the repeat base length was determined as 82-83 bp in *H. huso,* 84 bp in *A. stellatus*, and 82 bp in *A. gueldenstaedtii*. While the repeat sequences of *H. huso* and *A. stellatus* have a high degree of similarity, the repeat sequences for *A. gueldenstaedtii* were different (Figure 2).

Discussion

Previous phylogenetic and population genetics studies have been conducted on *Acipenser gueldenstaedtii*, *A. stellatus* and *Huso huso* species, as well as other sturgeon species, all of which are Eurasian species in the Black Sea [3-5,18,25]. However, there are no molecular or morphometric analysis based studies previously conducted on sturgeon fish in Turkey. In this study, by sampling from the Black Sea coasts of Turkey, and by performing sequence analysis of the mtDNA tRNA[pro]-Dloop gene region, comparisons were made both within this study and also with other studies.

Length variation and heteroplasmy

Most individuals of most vertebrate species possess a single form of mtDNA that is inherited from the female parent. Nevertheless, some individuals and some species exhibit more than one form of mtDNA known as heteroplasmy. This heteroplasmy level of variation has been demonstrated in most animal species including birds [26], mammals [27], frogs, *Rana esculenta,* [28] and humans [29]. It has also been reported in several cartilaginous and bony fish [3-6, 30]. There are large random shifts in heteroplasmy level between female parent and offspring. Understanding the distribution in heteroplasmy levels across a group of offspring is an important step in understanding the combination of haploidy and maternal inheritance of mtDNA in species.

In this study, size variants in homoplasmic and heteroplasmic sturgeon was examined and 9% of total individuals were found to be heteroplasmic with 2-6 different mtDNA size classes. Among three species, *A. stellatus* is the most heteroplasmic species with the frequency of 41.7%. When the heteroplasmy reported for certain fish species was reviewed, the distribution rates of heteroplasmic individuals within the population reached up to 5% in *Amia calva* (bowfins) [31], 12% in the Clupedae species *Alosa fallax* [10], and 42% in sturgeons [3,12]. Furthermore, Ludwig *et al.* [13] evaluated 1,238 samples including 19 species of sturgeon and 1 paddlefish, and calculated heteroplasmy rates as 8% for *H. huso,* 14.3% for *A. gueldenstaedtii*, 19.9% for *A. baerii*, 30% for *A. naccarii* and 25.8% for *A.*

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                                      tRNA^pro  ←|→ D-loop
                          |→   TAS   ←|
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
              10        20        30        40        50        60        70        80        90       100
A.guelden.  -ACCCTTAACCC-CCAAAGCTAAGATTCTACATTAAAACTATTCTCTGACTA--------------------------------------TACCACAATGGT
A.stellatus TACCCTTAACCT-CCAAAGCTAAGATTCTACATTAAAACTATTCTCTGACCATGCGTATGTTTAATCCACATTAATTTCTAGCCACCATAACATAA-TGC
H.huso      TACCTTTAACCCTCCAAAGCTAAGATTCTACATTAAAACTATTCTCTGACCACACCATGTTTTAACCCACACCAATTTCTAGCTGCCATACCATAAGTGT

              |→ TAS   ←|→                            Central Repeat                          ←|→  TAS
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
             110       120       130       140       150       160       170       180       190       200
A.guelden.  TTGCACGTACATTAAATTGTTTAAGTACATAAGGCATACTATGTT-TAATCCACATTAATTTCTAGCCACCATA--CCATAATGTTTGCACGTACATTAA
A.stellatus TTGCACATACATTAAATTATTTAGGTACATAAGACATGCTATGTT-TAATCCACATTAATTTCTAGCCACCATAAATTAAAATGCTTGCACATACATTAA
H.huso      TTACATATACATTAAATTATTCAAGTACATAAGACATGCTATGATATAATCCACATTAATTTCTAGCCACCATA--CCATAATGTTTACATATACATTAA

              ←|→                             CR                              ←|→   TAS ←|
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
             210       220       230       240       250       260       270       280       290       300
A.guelden.  ATTGTTTAAGTACATAAGGCATAC-TATGTTTAATCCACATTAATTTCTAGCCACCATA--CCATAATGTTTGCACGTACATTAAATTGTTTAA-GTACA
A.stellatus ATTAGTTAGGTACATAAGACATGCCTATGTTTAATCCACATTAATTTCTAGCCACCATAAATTAAAATGTTTCATCTAGCGTCAAATGAA----------
H.huso      ATTATTCAAGTACATAAGACATGC-TATGTATAATCCACATTAATTTCTAGCCACCATA--CCATAATGTTTACATATACATTAAATTATTCAA-GTACA

           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
             310       320       330       340       350       360       370       380       390       400
A.guelden.  TAAGGCATACCTATGTTTAATCCACATTAATTTCTAGCCACCATACCATAATGTTT-------------------------------------------
A.stellatus ----------------------------------------------------------------------------------------------------
H.huso      TAAGACATGC-TATGTATAATCCACATTAATTTCTAGCCACCATACCATAATGTT--------------------------------------------
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Figure 2. Alignment of sequences of partial tRNA^pro and D-loop of *A. gueldenstaedtii* (*A.g*), *A. stellatus* (*A.s*) and *H. huso* (*H.h*), (TAS motifs are in boldface and repeat units are underlined, tRNA^pro is italic).

stellatus. Differently from the study we have conducted, a maximum of 7 repeats were identified. However, this 7 repeat structure was observed in the *A. ruthenus* species, which was not included in our study due to anthropogenic impacts, Sterlet (*Acipenser ruthenus*), Atlantic sturgeon (*Acipenser sturio*) and Ship sturgeon (*Acipenser nudiventris*), which have nowadays almost disappeared from the Black Sea region.

The repeat region, responsible for length variations and heteroplasmy, is located near the 5' end of the D-loop and separated by only a few nucleotides from the tRNA gene [12] where replication is initiated, and the 3' end where the D-loop DNA is terminated [32]. Except certain fish species like the Paddlefish (*Polyodon spathula*) [4], these repeat units contain TAS motifs [12]. In addition, it was described that repeat units were associated with the TAS motifs. Interestingly, it was observed that areas beyond the TAS motifs were erased in most homoplasmic species. Intra and inter-specific mtDNA length variations in the control region, resulted by tandem repeats, have been observed in a number of fish species: cod (*Gadus morhua*) [9]; Redfishes (Sebastes: Scorpaenidae) [33]; Percid Fish Species (*Perca fluviatilis*, *Acerina cernua*, *Stizostedion lucioperca*) [34]; European sea bass (*Dicentrarchus labrax*) [35] and several sturgeon species (*Acipenser transmontanus*, *A. mikadoi*, *A. oxyrinchus*, *A. medirostris*, *A. ruthenus*, *A. sturio*, *A. baerii*, *A. fulvescens*, *A. gueldenstaedtii*, *A. naccarii*, *A. nudiventris*, *A. persicus*, *A. stellatus*, *A. transmontanus*, *A. brevirostrum* and *Huso huso* [3,4,12,36]. Aside from a few exceptions, it was determined that the central repeat unit's length varied between 74-83 bp, depending on the species. When identifying repeat units in this study, repeating base sequences were determined by taking

the central repeats into account. The length of repeating units was 83 bp for while the length of repeating units was 82 bp for *A. gueldenstaedtii*. However, Ludwig *et al*. [4] determined in their study a repeat of 82 bp for all three of the species. No consistency was observed between the repeat units. While the repeat for *H. huso* and *A. stellatus* were highly similar, differences were observed in the repeat unit sequences of *A. gueldenstaedtii*. In addition, a 36 bp deletion was only observed in the *A. gueldenstaedtii* species as Ludwig et al. [4] found.

The variation in mtDNA size present in the total sturgeon samples from Turkish waters was apportioned into hierarchical components as described in Rand and Harrison [23]. Gene diversity was calculated from the mtDNA size class frequencies by following the notation of Birky *et al*. [24]. We found very close and similar pattern of variation in the three species and polymorphic tandem repeat number within and among individuals. When gene diversity within individuals is estimated separately as length variation due to 2-6 repeats, genetic diversity within an individual (Kb) was higher in *A. gueldenstaedtii* than in *A. stellatus* and *H. huso (*0.625, 0.620 and 0.500, respectively). Genetic diversity within a species (Kc) was similarly high in the three species. Unlike the Kb values, the proportion of gene diversity within a species (GIP) is significantly lower for *A. gueldenstaedtii* (0.063) than for *A. stellatus* (0.203) and *H. huso* (0.285).

A number of authors have suggested that the main processes leading to heteroplasmy in homoplasmic lineages are mutation and paternal leakage [12, 23]. A likely mechanism which could generate length mutations is the insertion and deletion of repeats. This is an

improvement over models of slippage and mismatching of single strands during replication [37]. These mechanisms in homoplasmic lineages play an important role in the maintenance of heteroplasmy by increasing or decreasing copy number. Length heteroplasmy due to high mutation rate in mitochondrial control region requires several features: the presence of tandem repeats, a high degree of sequence identity among repeats, and the capability to form secondary structure when single stranded [36]. Another possibility is the contribution of paternal mtDNA, as observed in anchovy [6], in mice [27], in mussels [13] and in mammals [27]. Paternal mtDNA must proliferate considerably during embryogenesis, and to influence the evolution of populations, it will need to reach the germline. Paternal leakage converts variation between individuals into heteroplasmy, whereas mutations operate within lineages [3]. Most heteroplasmic sturgeons show changes in their variants which are increments of the copy number of the 2 or 6 repeats. Sequences of the tRNA^{pro}-D-loop region from three sturgeon species indicate the occurrence of both nucleotide polymorphisms in tandem repeat arrays and order shuffling between repeats. This situation might be considered comparable to the length variants observed in heteroplasmic sturgeons [3]. The above mentioned hypothesis for length heteroplasmy provides an acceptable explanation of this result.

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