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Complete mitochondrial DNA sequence of Persian sturgeon, *Acipenser persicus*: Mitogenome characterization and phylogenetic implications

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ABSTRACT

This study aimed to determine the complete mitochondrial genome of the Persian sturgeon. *Acipenser persicus*, Borodin 1897 by direct sequencing of PCR products to understand the systematic status of this species. The whole mitogenome sequence has been deposited in GenBank under accession number MW713795. The circular mitochondrial genome was 16,588 bp long and consisted of 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes, and one non-coding control region (D-loop). Besides, all genes were encoded on the heavy strain except for ND6 and eight tRNA genes, exhibiting high similarity with other vertebrates in mitochondrial gene arrangement. The overall nucleotide composition was 30.30 % A (a: 5026), 29.17 % C (c: 4839), 16.30 % G (g: 2702), 24.23 % T (4020: t), and a degenerate nucleotide (R), with a skewness of 55.5 % AT. This research characterized the termination-associated sequence domain (ETAS), two central conserved sequence block domains (CSB-E and CSB-F), and three conserved sequence block domains (CSB-1, CSB-2, and CSB-3) in the control region. It corroborates the regulatory elements of the D-loop, indicating similarities across the sturgeon control region to that of other fish, showing the homologous of the CSB-conserved blocks located upstream of these blocks. Moreover, results further supported the Persian sturgeon evolution by phylogenetic tree based on encoded H-strand 13 protein-coding genes of mitochondrial genomes of 23 related species. The results indicated that *A. persicus* is closely related to *Acipenser gueldenstaedtii* and forms a monophyletic group with *Acipenser sinensis*.

Keywords: *Acipenser persicus*, Persian sturgeon, mitochondrial genome, phylogenetic.
Article type: Research Article.

INTRODUCTION

Persian sturgeon, *Acipenser persicus* is an Acipenseriform fish and one of the anadromous Ponto-Caspians sturgeons (Acipenseridae) distributed in the Caspian basin, most abundant in the southern part. It is a demersal fish found in various habitats: marine, freshwater, and brackish waters (Kottelat & Freyhof 2007). The Persian sturgeon is subject to overexploitation and is critically endangered, considering the importance of the caviar and salted/smoked fillet trade (Adeli & Namdar 2015). The depletion of its stocks, like other sturgeons results from human disturbance in habitat degradation by water pollution and dam construction, leading to disconnected migration routes between the Caspian Sea and rivers (Nejat *et al.* 2018; Friedrich *et al.* 2019). In recent years, much effort has been made into fingerling production to restore the *A. persicus* population in the Caspian Sea basin and boost its aquaculture industry (Tavakoli *et al.* 2017). The complete mitochondrial DNA sequence of vertebrates is a circular molecule with a length of 16-19 kb, including 13 protein-coding genes, two ribosomal

RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and a control region (CR; Boore 1999). The significance of the mitochondrial genomic sequencing technique, integrated with bioinformatics, has been confirmed animal species identification (Yang *et al.* 2014). Moreover, mitochondrial genome studies achieved success in molecular evolution, population genetics, and phylogenetic analysis (Lee *et al.* 2007) due to its compact gene organization, fast evolutionary rate, maternal inheritance, and lack of genetic recombination (Miya *et al.* 2003; Inoue *et al.* 2009). Furthermore, previous studies demonstrated that the classification of genes for molecular evolution research is divided to poor, medium, and excellent for mitochondrial genes of each phylum (Rastorguev *et al.* 2008). This study focuses on the complete mtDNA genome in *A. persicus* deposited by authors in GenBank under accession number MW713795.1, aims to characterize the whole mitochondrial genome of *A. persicus* structure, find regulating motifs in D-loop, and explore the phylogenetic position of *A. persicus* in the phylogenetic tree of Acipenseridae based on 13 protein-coding genes of 23 sturgeon and paddlefish mitogenome.

MATERIALS AND METHODS

Samples and DNA extraction

The sample of *A. persicus* was collected from the International Sturgeon Research Institute breading station, Guilan, Iran. Following the manufacturer's protocol, total genomic DNA was extracted from the caudal fin. It was preserved in 95% ethanol using a DNeasy Blood & Tissue DNA extraction kit (Cat. No. / ID: 69504, Qiagen, Hilden, Germany) following the manufacturer's protocol.

Primer design, PCR amplification, and sequencing

A series of primers were designed according to the mitochondrial genome sequence of *Acipenser transmontanus* (GenBank AB042837.1), *Acipenser dabryanus* (GenBank KP981414.1), *Acipenser stellatus* (GenBank MK213064.2), *Acipenser gueldenstaedtii* (GenBank FJ392605.1), *Acipenser baerii* (GenBank JQ045341.1), *Acipenser sinensis* (GenBank EU719645.1) using NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The designed primers were used to amplify overlapping PCR fragments. The amplifications were performed in 25 μL reaction volume containing 2× Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark), ten picomole of each primer (Takapoozist, Tehran, Iran), approximately 100 nanograms of template genomic DNA. PCR was performed under the following conditions: denaturation at 95 °C for 5 min, followed by 33 cycles at 95 °C for 30 s, 50–58 °C for 45 s, and 72 °C for 1 min, as well as further incubation for 10 min at 72 °C. Subsequently, the PCR products were purified and directly sequenced by Macrogen Company (Seoul, South Korea) using the Sanger dideoxy sequencing method (Sanger *et al.* 1977).

Gene annotation and sequence analysis

The quality control of raw sequence was analyzed using Bioedit software (Hall *et al.* 1999; Alzohairy 2011) and aligned with the MAFTT program (https://mafft.cbrc.jp/alignment/software/). The Vector NTI Advance (V 11.5) software was run to edit and contig the raw sequences, and then a complete mitogenome sequence was obtained. The protein-coding genes (PCGs), ribosomal RNA genes, transfer RNA genes, and D-loop were annotated by MitoAnnotator software (Sato *et al.* 2018).

The FIMO part in MEME Suite 5.4.1 tools found the motifs in the control region sequences of D-loop (Bailey *et al.* 2009).

Phylogenetic analyses

A phylogenetic tree was constructed on the concatenated dataset of 13 Protein Coding Genes (PCGs), excluded Control region, at the nucleotide level with Maximum likelihood methods, the best nucleotide substitution model = GTR + G + I (general time-reversible) and 1000 replicates using Mega X (Kumar *et al.* 2018), to visualize the phylogenetic relationships between *A. persicus* and 23 other acipenserids (sturgeons and paddlefishes). The common carp was selected as an outgroup (accession number: OL699932.1).

RESULTS AND DISCUSSION

Mitochondrial genomic structure

We present the complete sequence of the mitochondrial genome of *A. persicus*, deposited in GenBank under the accession number MW713795. The identified haplotype of Persian sturgeon mitochondrial DNA was 16,588 bp in length, with the typical gene arrangement of vertebrate mtDNA (Miya *et al.* 2003), consisting of 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes, one displacement loop locus, and an origin of

replication on the light strand. All genes encoded on the heavy strain except for ND6 and eight tRNA genes, including $tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, $tRNA^{Ser}$ (UCN), $tRNA^{Glu}$, and $tRNA^{Pro}$ (Table 1 & Fig. 1).

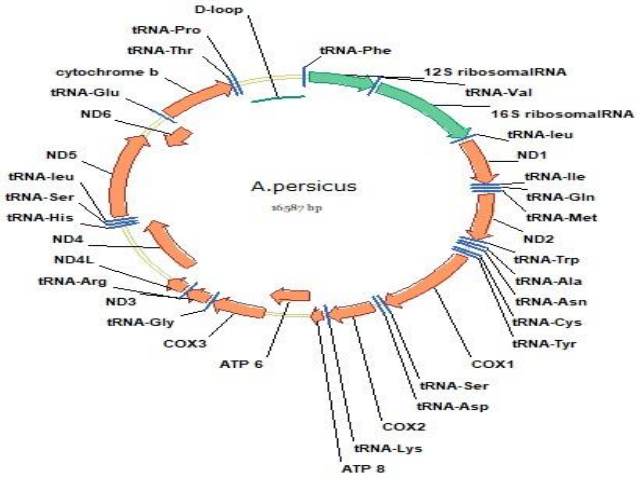

Fig. 1. The gene map of the complete mitochondrial genome of the *Acipenser persicus*. Clockwise and anticlockwise arrows indicate the direction of genes encoded on the H-strand and L-strand in the circular map of the mitogenome, respectively.

The gene size of *A. persicus* mitogenome is mainly similar to those found in other sturgeons, including *Acipenser* schrenckii (Li *et al.* 2014; Wang *et al.* 2015), *A. gueldenstaedtii* (Dong *et al.* 2016), *Huso dauricus* (Lu *et al.* 2016), and *A. ruthenus* (Li *et al.* 2015). However, mitogenomes represented a variable control region length due to sequence insertion or deletion changes (Sbisà *et al.* 1997). Twelve of the 13 protein-coding genes used ATG as the start codon except for COI (GTG). Eight protein-coding genes use TAG (ND1 and ND6) or TAA (COX1, ATP8, ATP6, COX3, ND4L, and ND5) as a stop codon, while five genes (ND2, COX2, ND3, ND4, and Cytb) end with an incomplete termination codon T (Table 1). In accordance with the incomplete stop codons in some of the *A. persicus* mitochondrial protein-coding genes, previous studies have demonstrated a common phenomenon in the teleost mitochondria (Chen *et al.* 2012; Chu *et al.* 2013). Post-transcriptional polyadenylation presumably completes these incomplete codons as TAA (Boore 1999). The overall nucleotide composition of *A. persicus* mitochondrial DNA was 30.30 % A (a: 5026), 29.17 % C (c: 4839), 16.30 % G (g: 2702), 24.23 % T (4020: t), and a degenerate nucleotide (R), with skewness of 55.5 % AT. The base composition in *A. persicus* mitochondrial genome, showing AT-richness, mirrors those of the previous studies, indicating a noticeable anti-guanine bias typically observed in fishes (Zhang *et al.* 2016; Islam *et al.* 2020).

Transfer RNA and ribosomal genes

Twenty-two tRNA genes were identified in A. persicus, showing the typical gene arrangement in most fishes and other sturgeons (Dong *et al.* 2016; Liu *et al.* 2017; Rocha-Reis *et al.* 2020). All tRNA genes interspersed between the rRNA and protein-coding genes like other fishes, with sizes ranging from 67 ($tRNA^{Ser}$) to 75 bp ($tRNA^{Leu}$), being identical to those in other sturgeons (Dong *et al.* 2016; Lu *et al.* 2016). The rRNAs, tRNAs, and protein-coding gene location details are presented in Table 1.

Table 1. Characteristics of the *A. persicus'* mitochondrial DNA genome.

	Gene name	strand	Start position	End position	Intergeneric Nucleotide	Overlapping nucleotides	Start codon	Stop codon	Nucleotide (bp)	Amino acid
1	$tRNA_{Phe}$	H	1	68					68	
2	12S rRNA	H	69	1029					961	
3	$tRNA_{Val}$	H	1030	1100					71	
4	16S rRNA	H	1101	2802					1702	

5	tRNA$_{leu}$	H	2803	2877					75	
6	ND1	H	2878	3852			ATG	TAG	975	324
7	tRNA$_{Ile}$	H	3862	3932	9				71	
8	tRNA$_{Gln}$	L	3933	4002					70	
9	tRNA$_{Met}$	H	4002	4071		1			70	
10	ND2	H	4072	5116			ATG	T--	1045	348
11	tRNA$_{Trp}$	H	5117	5189					73	
12	tRNA$_{Ala}$	L	5192	5260	2				69	
13	tRNA$_{Asn}$	L	5262	5334	1				73	
14	tRNA$_{Cys}$	L	5369	5435	34				67	
15	tRNA$_{Tyr}$	L	5436	5505					70	
16	COX1	H	5508	7061	2		GTG	TAA	1554	517
17	tRNA$_{Ser}$	L	7070	7138	8				69	
18	tRNA$_{Asp}$	H	7148	7219	9				72	
19	COX2	H	7234	7924	14		ATG	T--	691	230
20	tRNA$_{Lys}$	H	7925	7998					74	
21	ATP8	H	8000	8167	1		ATG	TAA	168	55
22	ATP6	H	8158	8841		10	ATG	TAA	684	227
23	COX3	H	8841	9626		1	ATG	TAA	786	261
24	tRNA$_{Gly}$	H	9626	9698		1			72	
25	ND3	H	9699	10046			ATG	T--	348	116
26	tRNA$_{Arg}$	H	10047	10116					70	
27	ND4L	H	10117	10413			ATG	TAA	297	98
28	ND4	H	10407	11787		7	ATG	T--	1381	460
29	tRNA$_{His}$	H	11788	11856					69	
30	tRNA$_{Ser}$	H	11857	11923					67	
31	tRNA$_{leu}$	H	11925	11997	1				73	
32	ND5	H	11998	13839			ATG	TAA	1842	613
33	ND6	L	13836	14357		4	ATG	TAG	522	173
34	tRNA$_{Glu}$	H	14358	14427					70	
35	CYTB	H	14431	15571	2		ATG	T--	1141	380
36	tRNA$_{Thr}$	H	15572	15644					73	
37	tRNA$_{Pro}$	L	15648	15717	3				70	
38	D-loop	H	15718	16588					871	

Note: H and L indicate genes transcribed on the heavy and light strands, respectively; bp: base pair.

The tRNA^{Val} gene separates two ribosomal RNA genes, a small subunit of rRNA (12S rRNA) and a large subunit of rRNA (16S rRNA) similar to other mitochondrial genes in animal mtDNA (Yang *et al.* 2014), with the length sizes of 961 bp and 1702 bp, respectively, both located between the tRNA^{Phe} and tRNA^{Leu} genes.

Non-coding regions

The control region (D-loop) was located between tRNA^{Pro} and tRNA^{Phe}, with 871 bp length. This finding differs from the earlier study, reporting the control region length of 870 bp in *A. persicus* (Dadkhah *et al.* 2019). In their study, the overall structure of the D-loop region showed that the sturgeon control region is similar to that of other fish, including the homologs of the CSB-conserved blocks, which are located upstream of these blocks. Results in the present study, characterized the termination-associated sequence domain (TAS), two central conserved sequence block domains (CSB-D and CSB-F), and three conserved sequence block domains (CSB-1, CSB-2, and CSB- 3) in the *A. persicus* control region (Fig. 2).

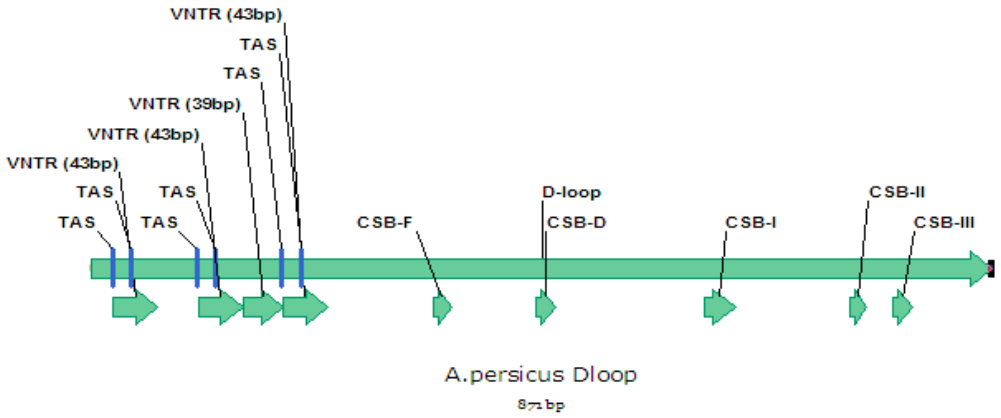

Fig. 2. Schematic structures of mitochondrial control regions in *A. persicus*. TAS, termination associated sequences; CCD, central conserved domain; CBSs, conserved sequence blocks.

It corroborates the regulatory elements of the D-loop, indicating similarities across the *A. persicus* control region to that of previous research in *A. persicus* and other sturgeons such as *A. gueldenstaedtii, A. stellatus, and A. nudiventris* (Dadkhah *et al.* 2019). Indeed, it is consistent with results of other fish species which exhibit the location of CSB-1, 2, and 3 upstream of the central conserved sequence block domains.

These CSBs (CSB-1, CSB-2, and CSB-3), the highly conserved and identified domains, play a role in the formation and positioning of RNA polymerase for transcription and initiating the H-strand DNA replication near the CSB- 1 (OH; Shadel & Clayton 1997). Moreover, the consensus central conserved sequences of CSB-1 (enriched in A and T), CSB-2, and CSB-3 (enriched in A and C) were ATAATGAATAGTGAA TGATATAATGACATA, CAAACCCCCTACCCCC, and TGTCAAACCCCAAAAGCA, respectively (Table 2).

Table 2. Base composition of each protein-coding gene in the *A. persicus* mitochondrial DNA.

	gene	Base composition (%)				G+C content (%)	Nucleotide (bp)
		A	T	G	C		
1	ND1	280	247	136	312	45.9	975
2	ND2	344	229	128	344	45.2	1045
3	COX1	384	437	300	433	47.2	1554
4	COX2	214	176	117	184	43.6	691
5	ATP8	55	44	20	49	41.1	168
6	ATP6	185	174	91	234	47.5	684
7	COX3	206	200	138	242	48.3	786
8	ND3	85	97	60	109	48.1	351
9	ND4L	72	73	44	108	51.2	297
10	ND4	408	336	206	431	46.1	1381
11	ND5	566	451	237	587	44.8	1841
12	ND6	61	217	184	60	46.7	522
13	CYTB	309	305	173	354	46.2	1141

Note: bp: base pair.

These results differ from those previously reported by Dadkhah *et al*. (2019), where only one motif of CSB-D and one TAS were detected. The central conserved domains (CCD) containing five blocks (CSB-B, CSB-C, CSB-D, CSB-E, and CSB-F) are found in mammals usually. However, only CSB-D, CSB-E, and CSB-F were primarily detected in fishes (Lee *et al.* 1995), exhibiting Chen iting little variability, thought to have critical functions in mitochondrial metabolism (Chen *et al.* 2004). While six (CSB-F to CSB-A) and three conserved sequence blocks (CSB-F, CSB-E, and CSB-D) were easily recognizable in the central conserved domain of *Nannostomus eques* and *N. unifasciatus* (Terencio *et al.* 2013) and 21 grouper species (Zhuang *et al.* 2013) respectively, in our study, CSB-E was not found in the control region. Moreover, we identified variable number tandem repeats (VNTR), repeating three times (43 bp) and one time (39 bp) along the D-loop (Fig. 3).

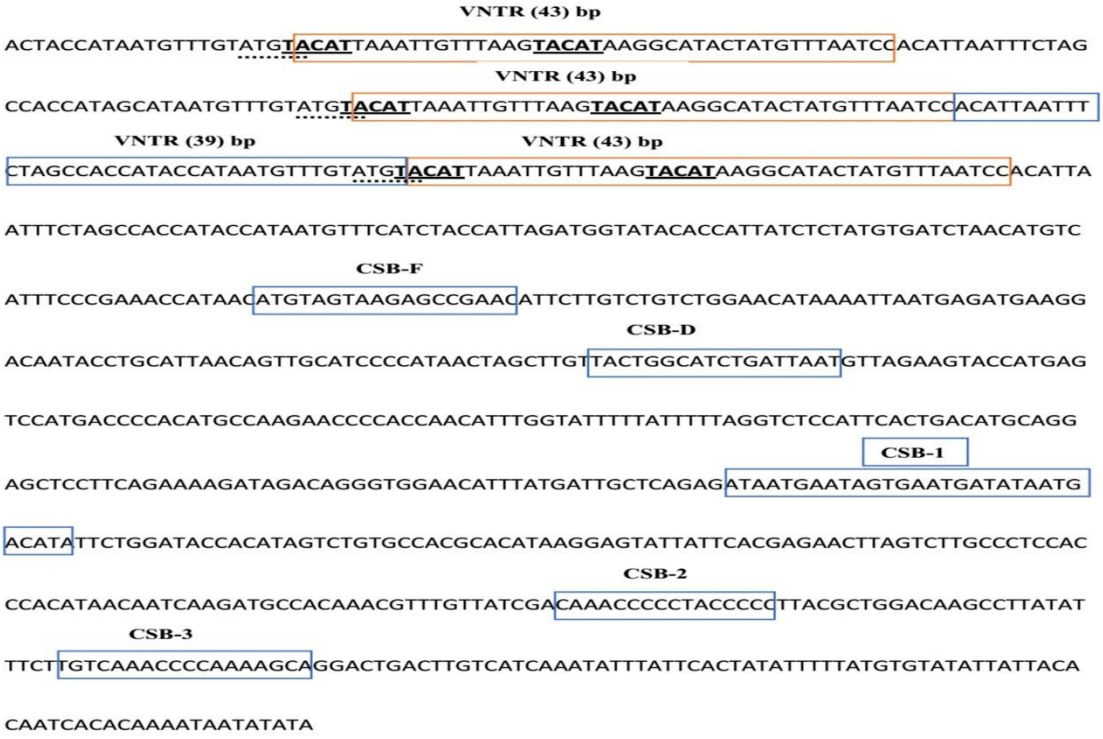

Fig. 3. The structure of control region in *A. persicus*. The dashed and simple lines depict the reversed complement cTAS (ATGTA) and TAS (TACAT) motifs, respectively. Central conserved sequence block domains (CSB-D and CSB-F), three conserved sequence block domains (CSB-1, CSB-2, and CSB-3), and the variable number of tandem repeats are in the boxes.

In teleost fish, VNTRs characterized by heteroplasmy are most frequently found in the ETAS (Ortí *et al.* 2008), playing a role in mtDNA variation and genetic marker (Chen *et al.* 2004). Six times repetition of TASs are conserved pentanucleotides 5′–TACAT—3′ and three reversed complement cTAS motif 3′-ATGTA-5′, included in the structure of VNTR (Figs. 2-3), which is in agreement with the previous study in *A. persicus* (Dadkhah *et al.* 2019).The TAS motif can pair with the cTAS motif to form stable hairpin loops generating sequence-specific signals, thought to terminate the D-loop replication in the heavy chain strand (Saccone 1991; Terencio *et al.* 2013).

Phylogenetic analysis

The phylogenies, based on the 13 protein-coding mitogenome genes of 24 sturgeons and paddlefish species, represent high bootstrap support (Fig. 4). The constructed maximum-likelihood tree indicated that the mitogenome related to *A. persicus* in this paper deposited in GenBank (MW713795.1) is most closely related to *A. gueldenstaedtii* and is in a monophyletic group with *A. sinensis*. This result is similar to that previously clarified in a phylogenetic tree in this manuscript, indicating that *A. sinesis* (NC_012646.1) with another gene bank deposited mitogenome was clustered with *A. gueldenstaedtii*. In the present study, different results were obtained from those of Liao *et al.* (2016), who claimed that two species, *A. sinesis* and *A. dabryanus* are closely different from each other. The clade is divided into paddlefish genera (*Polyodon* and *Psephurus*) and other sturgeons. The

genus *Scaphirhynchus*, inhabiting the Atlantic Ocean, is a sister group in the clade containing Ponto-Caspian species. The findings observed in this study mirror those of the previous one, indicating the position of this genus between Ponto-Caspian species in the ML analysis of the phylogenetic tree (Rastorguev *et al.* 2008).

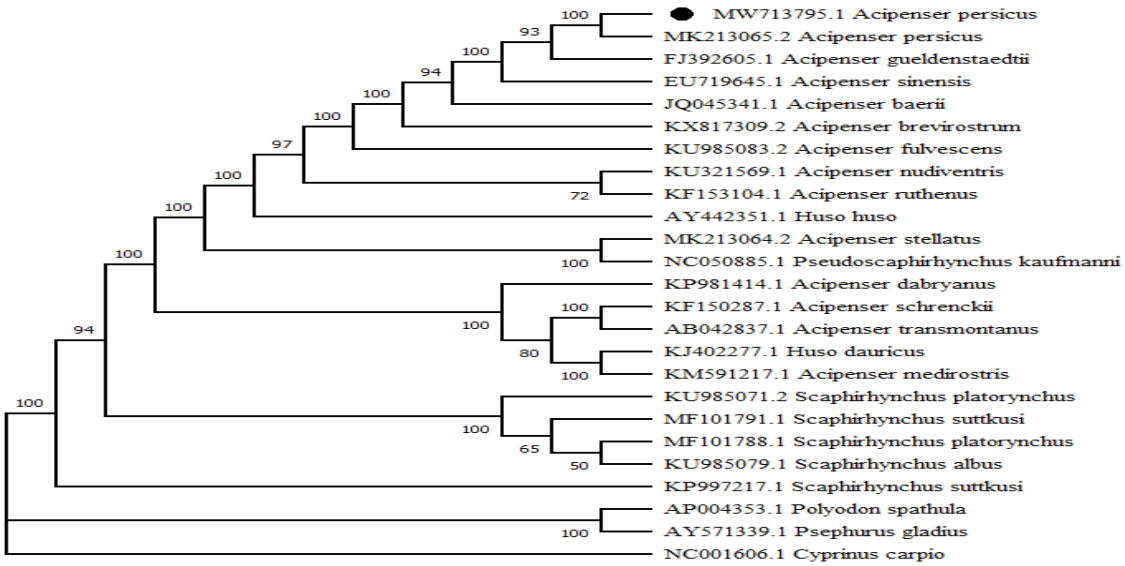

Fig. 4. Maximum-likelihood tree based on 13 mitochondrial protein-coding genes of sturgeon and paddlefish with (GTR+I+G) nucleotide evolution model. Numbers above the internal branches are bootstrap values after 1000 replications.

Moreover, in this phylogenetic analysis within the Ponto-Caspian clade, the genus *Huso* was classified as a sister group of the *Acipenser* genus, matching results reported in earlier studies (Krieger *et al.* 2008; Sheraliev & Peng 2020). These data provide useful information for a better understanding of *A. persicus* mitogenomic and genetic markers for identifying this species and studying population genetics.

CONCLUSION

The circular mitochondrial genome was 16,588 bp long and consisted of 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes, and one non-coding control region (D-loop). In the control region, the termination-associated sequence domain (ETAS), two central conserved sequence block domains (CSB-E and CSB-F), and three conserved sequence block domains (CSB-1, CSB-2, and CSB-3) were recognized. Persian sturgeon evolution was determined by phylogenetic tree based on encoded H-strand 13 protein-coding genes of mitochondrial genomes of 23 related species using MEGA X. The results indicated that *Acipenser persicus* is the most closely related to *A. gueldenstaedtii* and is in a monophyletic group with *A. sinensis*.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability statement

The data that support the findings of this study (*Acipenser persicus*) is available in GenBank at https://www.ncbi.nlm.nih.gov/, accession number: MW713795.1.

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