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Complete Mitogenome of the Russian Sturgeon *Acipenser gueldenstaedtii* (Acipenseriformes; Acipenseridae)

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Sturgeons and paddlefishes are frequently referred to as 'living fossils' among the actinopterygian lineage. They are increasingly facing threats to their existence because of various anthropogenic pressures. In this study, we present the complete mitogenome sequence of the Russian sturgeon *Acipenser gueldenstaedtii* (Acipenseriformes; Acipenseridae). The mitogenome showed highly homogeneous molecular features compared to previously known vertebrate mitogenomes. Phylogenetic tree inferred from concatenated protein-coding and tRNA genes unambiguously revealed the monophyly of *A. gueldenstaedtii*, *Acipenser stellatus*, and *Huso huso*. Genetic information of the endangered *A. gueldenstaedtii* will provide baseline data needed to develop molecular markers for stock identification and assessment of population diversity and also to develop future conservation strategies.

Key words: *Acipenser gueldenstaedtii*, Mitogenome, Phylogeny, Russian sturgeon

Introduction

Sturgeons and paddlefishes inhabit rivers, estuaries, nearshore oceans, and inland seas of the Northern Hemisphere (Billard and Lecointre, 2001). They have undergone remarkably little morphological changes and are frequently referred to as 'living fossils' in the actinopterygian lineage (Gardiner, 1984; Bemis et al., 1997). Sturgeons are distinctive not only for primary cartilaginous endoskeleton, but also for rows of bony scutes, sensory barbels, gill rakers, lack of teeth, flattened rostra, and heterocercal caudal fin (Bemis et al., 1997; Billard and Lecointre, 2001; Nelson, 2006). They have long life spans and achieve reproductive maturity late in life (Bemis et al., 1997; Billard and Lecointre, 2001). Some species of sturgeons have experienced repeated rounds of genome duplications (Birstein et al., 1997).

Classification of sturgeons have been challenged because of their morphological plasticity (see Bemis et al., 1997 for review), natural hybridization and introgression (Birstein, 1993; Bemis et al., 1997; Birstein et al., 1997; Billard and Lecointre, 2001), reduced evolutionary rate (Brown et al., 1996; Krieger and Fuerst, 2002), and endangered status (see below). The true number of species and subspecies still remains contentious (Birstein and Bemis, 1997). Recent molecular phylogenetic studies shed great insights into issues such as phylogenetic relationships (Ludwig et al., 2000, 2001; Peng et al., 2007), genome duplication events (Birstein et al., 1997; Ludwig et al., 2001; Peng et al., 2007), and biogeographic distributions (Ludwig et al., 2002, 2003; Birstein et al., 2005; Peng et al., 2007). Sturgeons are also distinct by possessing heterogeneous copies of 18S ribosomal RNA (rRNA) gene in the nuclear genome (Krieger et al., 2006) and a control region (D-loop) in the mitogenome (Brown et al., 1996; Ludwig et al., 2000) within an individual.

Over the last few decades, sturgeons and paddlefishes have paced numerous threats to their existence, not only from over-fishing and poaching, but also from water pollution and habitat degradation/fragmentation (Birstein, 1993; Billard and Lecointre, 2001; Pikitch et al., 2005). In fact, all extant species are listed as either 'vulnerable', 'endangered', or 'critically endangered' by the International Union for Conservation of Nature (IUCN). They are also included in the Convention on International Trade in Endangered Species (CITES) list which regulates their international trading. Worldwide conservation

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and restoration programs have been initiated by reducing harvest, tightening international trade regulations, restocking by captive breeding and reintroduction into the wild, and protecting natural habitat. Recent phylogeographic studies have great implications for such conservation and restoration initiatives (e.g., Ludwig et al., 2002, 2003; Tiedemann et al., 2007).

The order Acipenseriformes includes 25 valid extant species of sturgeons in the family Acipenseridae, which includes three genera (*Acipenser*, *Huso*, and *Scaphirhynchus*), and two extant species of paddlefishes in the family Polyodontidae, which includes two genera (*Polyodon spathula* and *Psephurus gladius*) (Birstein and Bemis, 1997). Among these acipenseriform species, mitochondrial genomes (mitogenomes) were fully sequenced from only seven species (Inoue et al., 2003; Peng et al., 2007). The complete, concatenated mitogenome sequences have been widely used to resolve phylogenetic relationships of diverge piscine taxa including ancient fish (Inoue et al., 2003; Miya et al., 2003; Arnason et al., 2004).

The Russian sturgeon *Acipenser gueldenstaedtii* is phylogenetically closely related to *A. baerii*, *A. naccarii*, and *A. persicus* (Ludwig et al., 2000, 2001; Peng et al., 2007), and the sturgeon species have complex population structures characterized by morphologically indistinguishable but genetically distinct cryptic lineages (Birstein et al., 2000; 2005). Several species of sturgeons are highly prized for their roe which is made into caviar, and also the taste of their meat. Captive rearing of sturgeons for aquaculture production of black caviar has been recently exploited in Korea with an increase in market demand, and *A. gueldenstaedtii* is one of the potential target species with this purpose (Birstein, 1993; Pikitch et al., 2005). In this study, we analyzed the complete mitogenome sequence of *A. gueldenstaedtii* as part of a baseline study for selecting molecular markers that can be used in stock identification, and also to conduct an assessment of population variability.

Materials and Methods

Fish sampling and genomic DNA extraction

Individuals of *A. gueldenstaedtii* used in this study were acquired from a local farm, Dinoville Aquafarm Inc. (Hamyang-gun, Korea), the strain of which were imported from Via Orizinuovi, Brescia, Italy. Genomic DNA was extracted from the barbel of an individual using the conventional SDS/proteinase K method followed by organic extraction and ethanol precipitation (Sambrook and Russell, 2001).

PCR, sequencing, and gene annotation

Five pairs of overlapping forward and reverse primers were designed in order to amplify the complete mitogenome sequence of *A. gueldenstaedtii* (Table 1). PCR runs in a 50-μL reaction volume included *ca.* 50 ng of genomic DNA, 1×Expand High Fidelity buffer with 1.5 mL $MgCl_2$, each 0.5 μM primer, 250 μM dNTP mix, and 2.6 units of Expand High Fidelity enzyme mix (Roche Applied Science, Mannheim, Germany). PCR was run with the following thermal cycling profile in iCycler (Bio-Rad, Foster City, CA, USA): an initial denaturation at 94°C for 2 min, 10 cycles of denaturation at 94°C for 15 s, annealing at 58°C for 30 s, and elongation at 68°C for 8 min, followed by 20 cycles of denaturetion at 94°C for 10 s, annealing at 58°C for 30 s, and elongation at 68°C for 8 min with 5 s increase for each successive cycle. The reaction was completed by a final elongation at 72°C for 7 min. The expected sizes of PCR amplicons ranged from 3.0 to 3.7 kb

Table 1. Information of primer pairs used to amplify the complete mitogenome of the Russian sturgeon *Acipenser gueldenstaedtii*

Primer	Sequence (5' to 3')[a]	Location	Expected size (kb)
Aspmt 1F	GCTAGCGTAGCTTAACTAAAGC	*trnF*	3.5
Aspmt 1R	CATATTGCTGCGAGGGGTCA	*nad1*	
Aspmt 2F	TCCGGTTGAGCCTCCAATTCA	*nad1*	3.7
Aspmt 2R	GTGGTTGTTAGTTCGACTGACAT	*cox1*	
Aspmt 3F	TTCCTAGGCCTCGCAGGAAT	*cox1*	3.7
Aspmt 3R	GCTGTTGTTGTGGTTCAAAGTC	*nad4*	
Aspmt 4F	TGCTACTAGCATTCTCRGCAT	*nad4L*	3.7
Aspmt 4R	TAAGGATYTGTCCTTGCTTCG	*nad6*	
Aspmt 5F	TCCTCCTCATCACAYTAATCTAA	*nad5*	3.0
Aspmt 5R	GTGCCTGATACCTGCTCCTTT	*rns*	

[a]Degenerate nucleotide bases are labeled according to IUPAC codes: R=A or G and Y=C or T.

(Table 1). The PCR products were purified with an *AccuPrep*® Gel Purification kit (Bioneer, Daejeon, Korea), cloned into pGEM®-T Easy Vector (Promega, Madison, WI, USA), and transformed into competent cells (*Escherichia coli* XL1-Blue MRF'; Stratagene, La Jolla, CA, USA). Three white *E. coli* colonies from each PCR product were picked out, and the plasmid DNAs were extracted using the alkaline lysis method (Sambrook and Russell, 2001). The three PCR clones were sequenced using ABI 3700 Automatic Sequencer (Applied Biosystems, Foster City, CA, USA) with two conserved vector primers and 20 sequencing primers. The consensus sequences were assembled in Sequencher™ (Gene Codes, Ann Arbor, MI, USA) to make the complete mitogenome sequence.

Annotation of protein-coding, rRNA, and transfer RNA (tRNA) genes, and determination of their gene boundaries of *A. gueldenstaedtii* were carried out with reference to mitogenome sequences of acipenseriform species publicly available in GenBank. Nucleotide base frequencies and codon usage of protein-coding genes were calculated in DAMBE (Xia and Xie, 2001). The synonymous mitochondrial gene labels of Boore (1999) were consistently used throughout this manuscript (Table 2). The complete mitogenome of *A. gueldenstaedtii* were deposited in GenBank under the accession number FJ392605.

Phylogenetic analysis

Complete mitogenome sequences of all available acipenseriform species were retrieved from GenBank. Sequences of fish species referred to as 'ancient fish' [i.e., a bowfin (the Amiiformes), gars (the Semionotiformes), and bichirs (the Polypteriformes)] were also downloaded for comparative phylogenetic analysis. They were aligned together with the *A. gueldenstaedtii* sequence in BioEdit 7.0.9 (Hall, 1999) and manually refined. Sequences of Light (L)-stranded genes were converted into complementary strand sequences. Nucleotide sequence alignment of protein-coding genes was created based on alignments of the corresponding proteins in DAMBE (Xia and Xie, 2001). Alignment of tRNA genes was carried out with reference to their secondary cloverleaf structure models predicted in tRNAscan-SE 1.21 (Lowe and Eddy, 1997), and *trnS2* was aligned with reference to a lancelet *Branchiostoma floridae* (Boore et al., 1999). Overlapping positions throughout mitochondrial genes were duplicated. Ambiguously aligned rRNA genes, *nad6* showing heterogeneous base composition, D-loop, and intergenic spacers were excluded in the final phylogenetic analysis, leaving 8,141 bp (including indels) for 12 protein-coding genes (without 3rd codon positions) and stem regions of 22 tRNA genes.

For phylogenetic analysis, two polypteriform species (*Erpetoichthys calabaricus* and *Polypterus ornatipinnis*) were used as outgroups. The nucleotide matrix was subjected to maximum likelihood (ML) analysis in PAUP* 4.0b10 (Swofford, 2002). Model selection strategy of Akaike Information Criterion (AIC) implemented in Modeltest 3.7 (Posada and Crandall, 1998) was used to determine the best-fit evolutionary model. ML tree was reconstructed using the TVM+I+Γ model with the likelihood settings, determined from Modeltest 3.7. The analysis was performed using the heuristic search option with random addition of sequences (10 replicates) and tree-bisection-reconnection branch swapping. Robustness of tree topologies was evaluated by bootstrap analysis with 1,000 pseudoreplicates.

Results and Discussion

Gene contents and arrangement

The complete mitogenome sequence of *A. gueldenstaedtii* is a circular molecule of 16,594 bp in total length (Fig. 1 and Table 2), which are similar to those of other vertebrates (Boore, 1999; Saccone et al., 1999). *A. gueldenstaedtii* possesses the gene contents and arrangement of typical vertebrate mitogenomes, which comprise 13 protein-coding genes for electron transport and oxidative phosphorylation, two rRNA genes, 22 tRNA genes, and D-loop. Twelve out of 13 protein-coding genes and 14 out of 22 tRNA genes are encoded on the Heavy (H)-strand, while *nad6* and eight tRNA genes (*trnQ*, *trnA*, *trnN*, *trnC*, *trnY*, *trnS1*, *trnE*, and *trnP*) are encoded on the L-strand.

Lengths of intergenic spacers vary to various extents (1 to 34 bp; Table 2). The largest of these is located downstream of *trnN* and upstream of *trnC*. The other gene boundaries are either abutted or overlapped. There are four notable overlappings between protein-coding genes *atp8* and *atp6* (10 bp), *atp6* and *cox3* (1 bp), *nad4L* and *nad4* (7 bp), and *nad5* and *nad6* (4 bp). The others are found between tRNA genes *trnI* and *trnQ* (1 bp) and *trnQ* and *trnM* (1 bp), and between protein-coding and tRNA genes *nad2* and *trnW* (1 bp), *cox3* and *trnG* (1 bp), and *nad3* and *trnR* (2 bp) in which the stop codons of protein-coding genes are overlapped by downstream sequence(s) of tRNA genes.

Table 2. Information on mitochondrial genes and noncoding region of the Russian sturgeon *Acipenser gueldenstaedtii*

Gene	Gene	Position[a]	Size (bp/aa)	Start codon	Stop codon	Strand[b]
tRNA Phe	*trnF*	1-68	68			H
Small subunit rRNA	*rns*	69-1,029	961			H
tRNA Val	*trnV*	1,030-1,100	71			H
Large subunit rRNA	*rnl*	1,101-2,802	1,702			H
tRNA Leu (UUC)	*trnL1*	2,803-2,877	75			H
NADH dehydrogenase 1	*nad1*	2,878-3,852 (+9)	975 (324)	ATG	TAG	H
tRNA Ile	*trnI*	3,862-3,932 (-1)	71			H
tRNA Gln	*trnQ*	3,932-4,002 (-1)	71			L
tRNA Met	*trnM*	4,002-4,071	70			H
NADH dehydrogenase 2	*nad2*	4,072-5,117 (-1)	1,046 (348)	ATG	TA	H
tRNA Trp	*trnW*	5,117-5,189 (+2)	73			H
tRNA Ala	*trnA*	5,192-5,260 (+1)	69			L
tRNA Asn	*trnN*	5,262-5,334 (+34)	73			L
tRNA Cys	*trnC*	5,369-5,435	67			L
tRNA Tyr	*trnY*	5,436-5,506 (+1)	71			L
Cytochrome *c* oxidase 1	*cox1*	5,508-7,061 (+8)	1,554 (517)	GTG	TAA	H
tRNA Ser (UCN)	*trnS1*	7,070-7,138 (+9)	69			L
tRNA Asp	*trnD*	7,148-7,219 (+14)	72			H
Cytochrome *c* oxidase 2	*cox2*	7,234-7,924	691 (230)	ATG	T	H
tRNA Lys	*trnK*	7,925-7,998 (+1)	74			H
ATP synthase 8	*atp8*	8,000-8,167 (-10)	168 (55)	ATG	TAA	H
ATP synthase 6	*atp6*	8,158-8,841 (-1)	684 (227)	ATG	TAA	H
Cytochrome *c* oxidase 3	*cox3*	8,841-9,626 (-1)	786 (261)	ATG	TAA	H
tRNA Gly	*trnG*	9,626-9,698	73			H
NADH dehydrogenase 3	*nad3*	9,699-10,049 (-2)	351 (116)	ATG	TAG	H
tRNA Arg	*trnR*	10,048-10,117	70			H
NADH dehydrogenase 4L	*nad4L*	10,118-10,414 (-7)	297 (98)	ATG	TAA	H
NADH dehydrogenase 4	*nad4*	10,408-11,788	1,381 (460)	ATG	T	H
tRNA His	*trnH*	11,789-11,857	69			H
tRNA Ser (AGY)	*trnS2*	11,858-11,925	68			H
tRNA Leu (CUN)	*trnL2*	11,926-11,998	73			H
NADH dehydrogenase 5	*nad5*	11,999-13,840 (-4)	1,842 (613)	ATG	TAA	H
NADH dehydrogenase 6	*nad6*	13,837-14,358	522 (173)	ATG	TAG	L
tRNA Glu	*trnE*	14,359-14,428 (+2)	70			L
Cytochrome *b* apoenzyme	*cob*	14,431-15,571	1,141 (380)	ATG	T	H
tRNA Thr	*trnT*	15,572-15,644 (+3)	73			H
tRNA Pro	*trnP*	15,648-15,717	70			L
Control region	D-loop	15,718-16,594	877			H

[a]The number in parenthesis indicates nucleotide base(s) of intergenic spacer (as the positive number) or overlapping (as the negative number).
[b]H and L indicate genes transcribed in the Heavy- and Light-strands, respectively.

Protein-coding genes

Thirteen mitochondrial protein-coding genes of *A. gueldenstaedtii* encode 55 (*atp8*) to 613 aa (*nad5*) (Table 2). Fig. 2 shows nucleotide base frequencies of protein-coding genes. In H-stranded genes, there is no notable bias at 1st codon positions, but there is bias against purines (A+G) (32.1%) toward pyrimidines (C+U) at 2nd codon positions. Anti-G bias (7.3%) toward A+C is remarkable at 3rd codon positions, which are free from selective constraints on nucleotide substitutions. In contrast, L-stranded *nad6* shows heterogeneous base composition with strong bias against A+C toward G+U at 1st and 3rd codon positions (19.6% and 16.7%, respectively). U is the most frequent base at 2nd codon positions (46.2%). These characteristics of nucleotide base frequencies at each codon positions in *A. gueldenstaedtii* are quite homogeneous to other acipenseriform species (Fig. 2) as well as other vertebrates including fish (e.g., Tzeng et al., 1992; Chang et al., 1994; Broughton et al., 2001).

All protein-coding genes but one use the canonical ATG start codon in *A. gueldenstaedtii* (Table 2). The exceptional *cox1* utilizes GTG for transcription initiation, which has been often reported as an alternative start codon in fish species (Tzeng et al.,

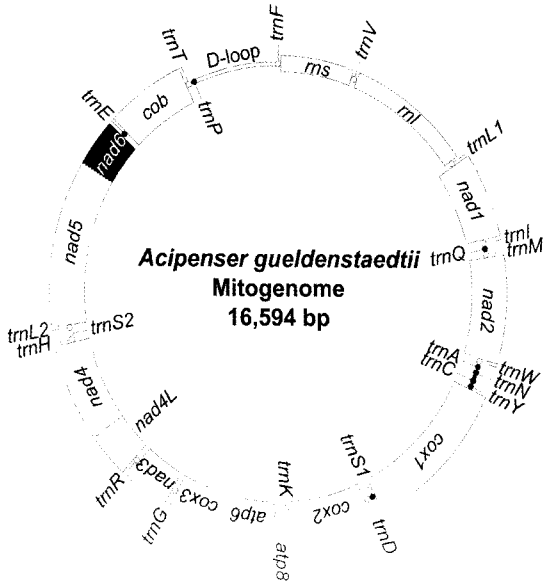

Fig. 1. Gene contents and arrangement of the mitogenome of the Russian sturgeon *Acipenser gueldenstaedtii*. Protein-coding genes are indicated by wide rectangulars, ribosomal RNA genes by narrow rectangulars, transfer RNA genes by circles, and noncoding control and interspacer regions by thick lines. Heavy- and Light-stranded genes or regions are indicated as empty and solid shapes, respectively. Overlappings between protein-coding genes are marked with wavy lines.

1992; Lee and Kocher, 1995; Boore et al., 1999). Nine protein-coding genes terminate with the stop codon, TAA or TAG, while *nad2*, *cox2*, *nad4*, and *cob* end with an incomplete T or TA, which is completed by addition of 3' A residue(s) to mRNA during post-transcriptional polyadenylation (Anderson et al., 1981; Ojala et al., 1981).

Genetic codes of mitochondrial protein-coding genes in *A. gueldenstaedtii* are shown in Table 3. The most frequently used codons are those with matching tRNA anticodons except Thr, Met, Ile, Pro, Ala, and Ser (*trnS1*). Patterns of codon usage of protein-coding genes show that twofold degenerate pyrimidine codons (NNY synonymous codon families) preferentially end with C to U, and most of twofold degenerate purine (NNR codon families) and fourfold degenerate codons (NNN codon families) end with A. In accordance with the anti-G bias of base frequencies, G is the least used nucleotides in 3rd codon positions in all NNN and NNR codon families, except Arg and Gly. These characteristics of codon usage in *A. gueldenstaedtii* are well congruent with other acipenseriform species (Table 3), as well as other vertebrates including fish (Broughton et al., 2001; Wang et al., 2007).

rRNA and tRNA genes

The mitogenome of *A. gueldenstaedtii* contains typical *rns* and *rnl* genes. Their occurrence is flanked by *trnF* and *trnL1*, and interposed by *trnV* (Fig. 1 and Table 2). The two rRNA genes are 961 and 1,702 bp in total lengths, respectively. Twenty-two tRNA genes of *A. gueldenstaedtii* intersperse between protein-coding and rRNA genes, either singly or in groups (Fig. 1 and Table 2). Lengths of tRNA genes vary from 67 (*trnC*) to 75 bp (*trnL1*). All tRNA genes are predicted to have the standard cloverleaf structures with normal base pairings including the G-U wobble except *trnS2* as in other fish species (Chang et al., 1994; Zardoya et al., 1995; Boore et al., 1999). They harbor identical anitcodons to other vertebrate genetic codes (Table 3).

Noncoding regions

A noncoding control region, D-loop of *A. gueldenstaedtii*, which contains initiation and promoter sites for H- and L-strand transcriptions (Doda et al., 1981; Clayton, 1991), is placed between *trnP* and *trnF*, and is 877 bp in length (Fig. 1 and Table 2). Despite its rapid evolutionary rate, several regulatory elements are relatively well conserved; they include conserved sequence blocks (CSBs) and termination-associated sequence (TAS) motifs (data not shown).

The site for origin of L-strand replication (O_L) occurs among five tRNA gene cluster, the WANCY region, between *nad2* and *cox1*. The intergenic region between the first three and the last two tRNA genes folds into the stable hairpin stem-loop structure consisting of a 12 bp-paired stem and a loop of 13 bp (data not shown) as other vertebrates (e.g., Zardoya et al., 1995), indicating its functional importance in L-strand replication mechanism. The consensus motif (5'-GCCGG-3') which represents a signal for transition from RNA to DNA synthesis in vertebrate mitogenomes is also identified at the base of the stem within *trnC*.

Phylogenetic analysis

Fig. 3 shows the phylogenetic tree of ancient fish taxa reconstructed by ML method with mitochondrial nucleotide sequence matrix from concatenated 12 protein-coding genes and stem regions of 22 tRNA genes. The phylogenetic tree recovers the strongly supported monophyly of acipenseriform species (100% bootstrap value) with respect to other ancient fish species; bichirs, bowfin, and gars. Within this lineage, the paddlefishes *P. spathula* and *P. gladius*

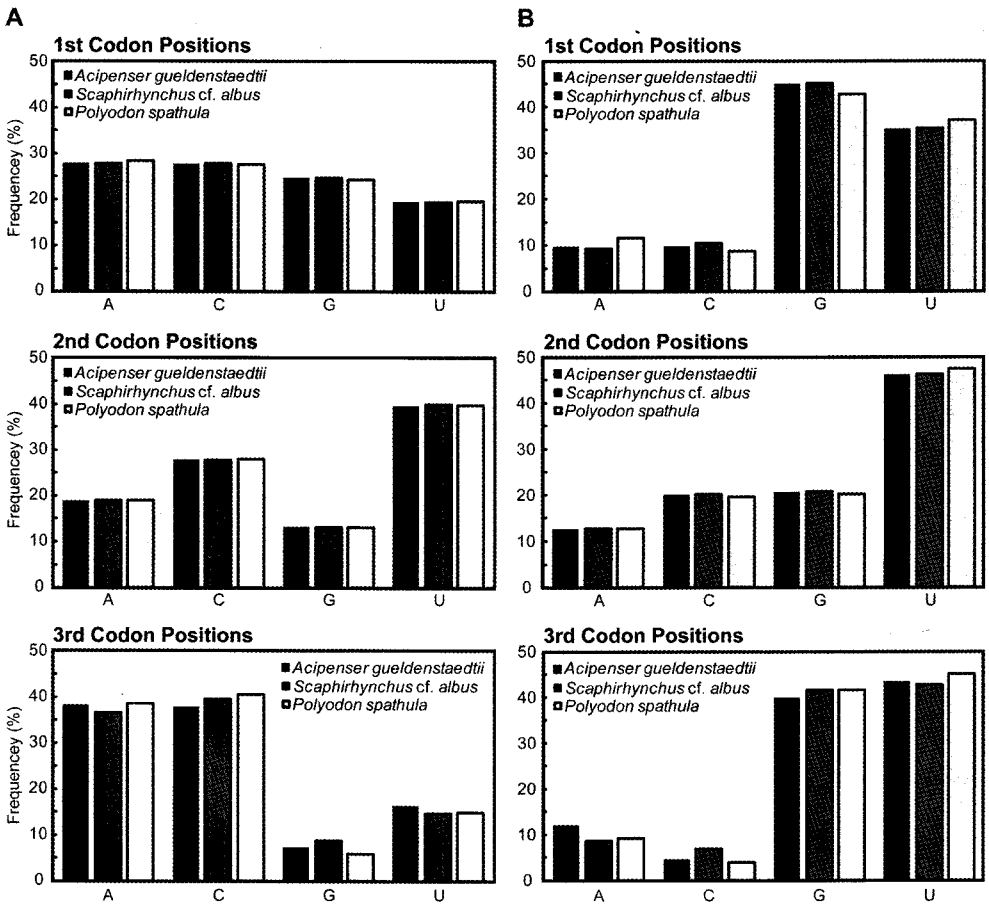

Fig. 2. Nucleotide base frequencies of mitochondrial protein-coding genes of three representative acipenseriform species. A) 12 protein-coding genes encoded on Heavy-strand and B) *nad6* encoded on Light-strand.

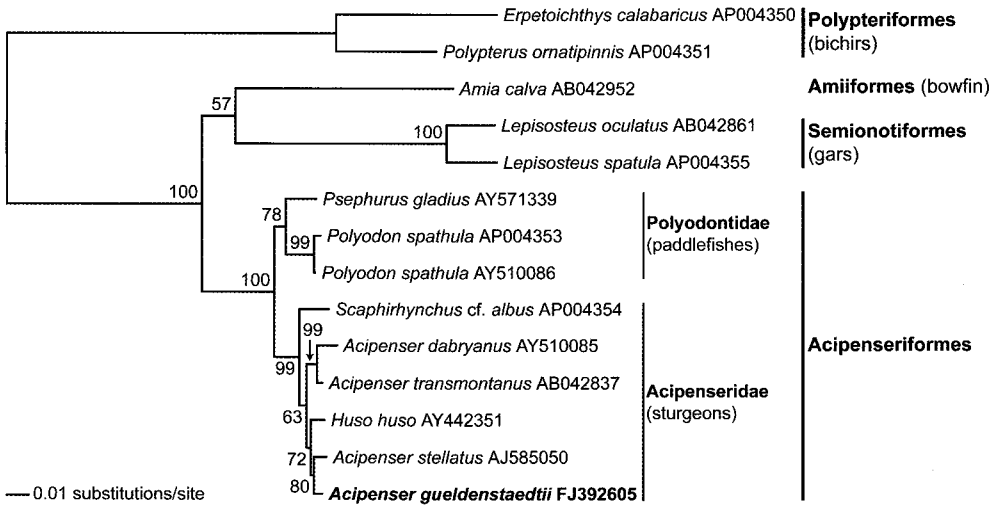

Fig. 3. Maximum likelihood (ML) tree of acipenseriform species inferred from the concatenated mitogenome sequence composed of 12 protein-coding (without 3rd codon positions and *nad6*) and stem regions of 22 tRNA genes. Two species of the Polypteriformes were used as outgroups. Ancient fish species of the Amiiformes and Semionotiformes were included for comparative phylogenetic analysis. Bootstrap value above 50% in ML analysis is shown at each branch node. Taxonomic placement is indicated for each taxon, and the Russian sturgeon *Acipenser gueldenstaedtii* analyzed in this study is boldfaced.

Table 3. Genetic codes and codon usage categorized into codon families of mitochondrial protein-coding genes of three representative acipenseriform species

Amino acid	tRNA gene	Anticodon	Codon [a]	Number (%) [b]		
				Acipenser gueldenstaedtii	*Scaphirhynchus* cf. *albus*	*Polyodon spathula*
Lys	*trnK*	UUU	AAA*	72 (1.9)	71 (1.9)	75 (2.0)
			AAG	7 (0.2)	8 (0.2)	4 (0.1)
Asn	*trnN*	GUU	AAC*	98 (2.6)	102 (2.7)	105 (2.8)
			AAU	32 (0.8)	28 (0.7)	32 (0.8)
Thr	*trnT*	UGU	ACA*	123 (3.2)	121 (3.2)	124 (3.3)
			ACG	14 (0.4)	19 (0.5)	10 (0.3)
			ACC	141 (3.7)	137 (3.6)	155 (4.1)
			ACU	34 (0.9)	36 (0.9)	31 (0.8)
Stop			AGA	0 (0.0)	0 (0.0)	0 (0.0)
			AGG	0 (0.0)	0 (0.0)	0 (0.0)
Ser	*trnS2*	GCU	AGC*	41 (1.1)	44 (1.2)	39 (1.0)
			AGU	10 (0.3)	6 (0.2)	11 (0.3)
Met	*trnM*	CAU	AUA	117 (3.1)	107 (2.8)	123 (3.2)
			AUG*	58 (1.5)	65 (1.7)	55 (1.4)
Ile	*trnI*	GAU	AUC*	135 (3.5)	155 (4.1)	173 (4.5)
			AUU	148 (3.9)	129 (3.4)	113 (3.0)
Gln	*trnQ*	UUG	CAA*	98 (2.6)	94 (2.5)	94 (2.5)
			CAG	6 (0.2)	11 (0.3)	8 (0.2)
His	*trnH*	GUG	CAC*	80 (2.1)	85 (2.2)	86 (2.3)
			CAU	22 (0.6)	17 (0.4)	17 (0.4)
Pro	*trnP*	UGG	CCA*	83 (2.2)	88 (2.3)	81 (2.1)
			CCG	13 (0.3)	8 (0.2)	4 (0.1)
			CCC	93 (2.4)	91 (2.4)	102 (2.7)
			CCU	30 (0.8)	26 (0.7)	31 (0.8)
Arg	*trnR*	UCG	CGA*	49 (1.3)	44 (1.2)	53 (1.4)
			CGG	8 (0.2)	11 (0.3)	3 (0.1)
			CGC	15 (0.4)	15 (0.4)	9 (0.2)
			CGU	4 (0.1)	5 (0.1)	9 (0.2)
Leu	*trnL1*	UAG	CUA*	259 (6.8)	241 (6.3)	253 (6.6)
			CUG	60 (1.6)	84 (2.2)	52 (1.4)
			CUC	128 (3.4)	131 (3.4)	139 (3.6)
			CUU	76 (2.0)	79 (2.1)	79 (2.1)
Glu	*trnE*	UUC	GAA*	83 (2.2)	76 (2.0)	84 (2.2)
			GAG	18 (0.5)	25 (0.7)	17 (0.4)
Asp	*trnD*	GUC	GAC*	57 (1.5)	59 (1.5)	52 (1.4)
			GAU	18 (0.5)	16 (0.4)	20 (0.5)
Ala	*trnA*	UGC	GCA*	110 (2.9)	101 (2.6)	109 (2.9)
			GCG	15 (0.4)	19 (0.5)	13 (0.3)
			GCC	176 (4.6)	173 (4.5)	163 (4.3)
			GCU	38 (1.0)	39 (1.0)	49 (1.3)
Gly	*trnG*	UCC	GGA*	89 (2.3)	91 (2.4)	102 (2.7)
			GGG	44 (1.2)	43 (1.1)	34 (0.9)
			GGC	84 (2.2)	92 (2.4)	82 (2.1)
			GGU	26 (0.7)	20 (0.5)	18 (0.5)
Val	*trnV*	UAC	GUA*	89 (2.3)	83 (2.2)	74 (1.9)
			GUG	40 (1.0)	46 (1.2)	36 (0.9)
			GUC	47 (1.2)	59 (1.5)	55 (1.4)
			GUU	46 (1.2)	35 (0.9)	47 (1.2)
Stop			UAA	10 (0.3)	9 (0.2)	10 (0.3)
			UAG	3 (0.1)	4 (0.1)	3 (0.1)
Tyr	*trnY*	GUA	UAC*	72 (1.9)	83 (2.2)	79 (2.1)
			UAU	46 (1.2)	35 (0.9)	38 (1.0)
Ser	*trnS1*	UGA	UCA*	62 (1.6)	60 (1.6)	59 (1.5)
			UCG	9 (0.2)	8 (0.2)	10 (0.3)
			UCC	81 (2.1)	87 (2.3)	78 (2.0)
			UCU	29 (0.8)	30 (0.8)	33 (0.9)
Trp	*trnW*	UCA	UGA*	102 (2.7)	96 (2.5)	110 (2.9)
			UGG	16 (0.4)	22 (0.6)	10 (0.3)
Cys	*trnC*	GCA	UGC*	18 (0.5)	19 (0.5)	22 (0.6)
			UGU	10 (0.3)	8 (0.2)	7 (0.2)
Leu	*trnL2*	UAA	UUA*	76 (2.0)	75 (2.0)	76 (2.0)
			UUG	26 (0.7)	25 (0.7)	30 (0.8)
Phe	*trnF*	GAA	UUC*	116 (3.0)	122 (3.2)	143 (3.7)
			UUU	105 (2.8)	97 (2.5)	82 (2.1)
				3,815 (100.0)	3,815 (100.0)	3,815 (100.0)

[a]Codons for which tRNAs with matching anticodons occur in mitochondrion are marked with an asterisk.
[b]Total number of each type of codon found among 13 protein-coding genes and frequency (%) of codon usage in parenthesis.

are genetically well separated from sturgeons *Scaphirhynchus* cf. *albus*, *Huso huso*, and four *Acipenser* species. Among the sturgeons, *S.* cf. *albus* holds the most basal position. Thereafter, *A. gueldenstaedtii* forms the strongly supported monophyletic group with *Acipenser stellatus* and *H. huso*, which inhabit the Atlantic regions, and are clearly separated from *Acipenser transmontanus* and *Acipenser dabryanus*, which inhabit the Pacific regions. *A. gueldenstaedtii* is placed at the terminal position with the closest phylogenetic affiliation to *A. stellatus*.

Phylogenetic bifurcation between polyodontid and acipenserid species, and placement of *S.* cf. *albus* prior to the Atlantic and Pacific clades among acipenserid species are well congruent with a previous study (Peng et al., 2007). Recent phylogenetic studies presented that species within the genera *Acipenser* and *Huso* clustered according to their geographical ranges (i.e., the Atlantic and Pacific clades) rather than taxonomic assignments (Ludwig et al., 2000, 2001; Peng et al., 2007). Our phylogenetic tree also shows that *A. gueldenstaedtii* forms a monophyletic group with the Atlantic-originated *A. stellatus* and *H. huso*, and genetically well separated from the Pacific originated *A. dabryanus* and *A. transmontanus*.

In this study, molecular structure of the mitogenome from the endangered but commercially important sturgeon *A. gueldenstaedtii* was determined along with its phylogenetic implications. Findings of this study not only add to our understanding of sturgeon phylogeny, but also offer baseline data with which to develop molecular markers for stock identification and population assessment as part of future conservation strategies.

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