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TECHNICAL NOTE

Characterization of the complete mitochondrial genome and phylogenetic analysis of *Pelodiscus sinensis*, a mutant Chinese soft-shell turtle

Jun Zhang[1] · Qingyan Zhou[1] · Xiaoli Yang[1] · Peng Yu[1,2,3] · Weishang Zhou[1] · Yuan Gui[1] · Xia Ouyang[1] · Quan Wan[1]

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Abstract

The Chinese soft-shell turtle (*Pelodiscus sinensis*, Testudines: Pelodiscus) shows geographical variation, and one strain is the inked turtle. Wild population numbers have dropped substantially during the past decades, and the species is now classed as vulnerable. However, little genetic data exists so this study aimed to sequence and analyze the complete mitochondrial genome. The circular double-stranded genome is 17,145 bp in length and contains 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes, an L-strand replication origin and a control region. The base composition is 35.5% A, 27.3% T, 11.8% G and 25.4% C, with an AT content of 62.8%. Trionychidae species were divided into two clades based on phylogenetic analysis, and the closest genetic distance was between *Trionyx axenaria* and *P. sinensis*. This study provides basic genetic data for future studies on conservation biology, phylogenetic and evolutionary analysis of this inked strain of the Chinese soft-shell turtle.

Keywords *Pelodiscus sinensis* · Soft-shell turtle · Mitochondrial genome · Phylogeny

The Chinese soft-shell turtle (*Pelodiscus sinensis*, Testudines: Trionychidae) is an important aquaculture species in southern China. However, wild population numbers have recently undergone a rapid decrease due to heavy hunting and deterioration of their environment, and today they are classed as a vulnerable (VU) IUCN species (http://www.iucnredlist.org/details/39620/0). The inked turtle strain, which is distributed in the middle and lower reaches of the Yangtze River, is a geographical variation: compared with other soft-shell turtle strains, the main feature of these turtles is the ink color of the whole body since its birth.

The mitochondrial genome is an efficient molecular marker widely used in analysis of population structure and genetic diversity (Sun et al. 2017; Tokishita et al. 2017) as well as species identification (Krzywinski et al. 2011) and phylogenetic analysis (Yu et al. 2016a, b). Knowing the complete mitochondrial genome would allow further genetic research and conservation (Feng et al. 2017; Jiang et al. 2017) of this threatened population.

In this study, we determined the complete mitochondrial genome sequence of a mutant *P. sinensis*. The specimen was collected from a location typical of the species (Wuhu City, Anhui Province, China). Total DNA was extracted from the muscles using the Ezup Column Animal Genomic DNA Kit (Sangon, Shanghai, China). The primers were designed based on known *P. sinensis* mitochondrial genome sequences (GenBank accession nos. AY687385.1, AY962573.1 and NC_006132.1). Sequences were assembled using DNASTAR software (DNASTAR, Madison, WI, USA) (Burland 2000). The newly-determined sequence was deposited into GenBank (accession no. MG431983).

The complete mitochondrial genome was found to be a circular double-stranded molecule 17,145 bp in length containing 22 tRNA genes, 13 protein-coding genes (PCGs), two rRNA genes, an origin of replication (O_L) and a control region (CR) (Table 1; Fig. 1a).

✉ Quan Wan
ahwanquan@163.com

1 College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, Anhui, China

2 State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

3 University of Chinese Academy of Sciences, Beijing 100049, China

Table 1 Annotation of the mutant *Pelodiscus sinensis* mitochondrial genome

Feature	Abbreviations	Position	Size nucleotide (bp)	Start codon	Stop codon	Aminoacid	Anti-codon	Intergenic nucleotide[a]	Strand[b]
tRNA^{Phe}	trnF	1–69	69				GAA	0	H
12S rRNA	12S	70–1048	979					0	H
tRNA^{Val}	trnV	1049–1118	70				TAC	−2	H
16S rRNA	16S	1117–2720	1604					0	H
tRNA$^{Leu(UUR)}$	trnL	2721–2797	77				TAA	0	H
ND1	nd1	2798–3769	972	ATG	TAG	323		−1	H
tRNA^{Ile}	trnI	3769–3838	70				GAT	−1	H
tRNA^{Gln}	trnQ	3838–3908	71				TTG	9	L
tRNA^{Met}	trnM	3918–3986	69				CAT	0	H
ND2	nd2	3987–5027	1041	ATG	TAG	346		−2	H
tRNA^{Trp}	trnW	5026–5098	73				TCA	11	H
tRNA^{Ala}	trnA	5110–5178	69				TGC	1	L
tRNA^{Asn}	trnN	5180–5253	74				GTT	0	L
OL		5254–5284	31					0	–
tRNA^{Cys}	trnC	5285–5349	65				GCA	0	L
tRNA^{Tyr}	trnY	5350–5415	66				GTA	1	L
COX I	cox1	5417–6961	1545	GTG	AGA	514		−5	H
tRNA$^{Ser(UCN)}$	trnS	6957–7027	71				TGA	1	L
tRNA^{Asp}	trnD	7029–7097	69				GTC	0	H
COX II	cox2	7098–7784	687	ATG	TAA	228		1	H
tRNA^{Lys}	trnK	7786–7858	73				TTT	1	H
ATP8	atp8	7860–8024	165	ATG	TAA	54		−10	H
ATP6	atp6	8015–8698	684	ATG	TAA	227		−1	H
COX III	cox3	8698–9481	784	ATG	T	261		0	H
tRNA^{Gly}	trnG	9482–9551	70				TCC	0	H
ND3	nd3	9552–9903	352	ATG	ATG	116		0	H
tRNA^{Arg}	trnR	9902–9971	70				TCG	0	H
ND4L	nd4L	9972–10,268	297	ATG	TAA	98		−7	H
ND4	nd4	10,262–11,647	1386	ATG	TAA	461		−5	H
tRNA^{His}	trnH	11,643–11,712	70				GTG	0	H
tRNA$^{Ser(AGY)}$	trnS	11,713–11,774	62				GCT	−1	H
tRNA$^{Leu(CUN)}$	trnL	11,774–11,848	75				TAG	0	H
ND5	nd5	11,849–13,627	1779	ATG	TAA	592		−5	H
ND6	nd6	13,623–14,147	525	ATG	AGG	174		0	L
tRNA^{Glu}	trnE	14,148–14,215	68				TTC	3	L
Cyt b	cytb	14,219–15,358	1140	ATG	TAA	379		3	H
tRNA^{Thr}	trnT	15,362–15,435	74				TGT	14	H
tRNA^{Pro}	trnP	15,450–15,520	71				TGG	0	L
Control region	CR	15,521–17,145	1625						–

[a]Negative intergenic nucleotides indicate overlapping sequences between adjacent genes

[b]H indicate heavy strand, L indicate light strand

Overall, the base composition was 35.5% A, 27.3% T, 11.8% G and 25.4% C, with an AT content of 62.8%. All genes were encoded on the heavy strand (H) except for one protein-coding gene (ND6) and eight tRNA genes (tRNA^{Gln}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys}, tRNA^{Tyr}, tRNA$^{Ser(UCN)}$, tRNA^{Glu} and tRNA^{Pro}) which were organized in a slightly shorter cluster on the light strand (L). Similar to other turtles (Amer and Kumazawa 2009; Jungt et al. 2006), 12S rRNA and 16S rRNA were separated by the tRNA^{Val} gene. The lengths of 12S rRNA and 16S

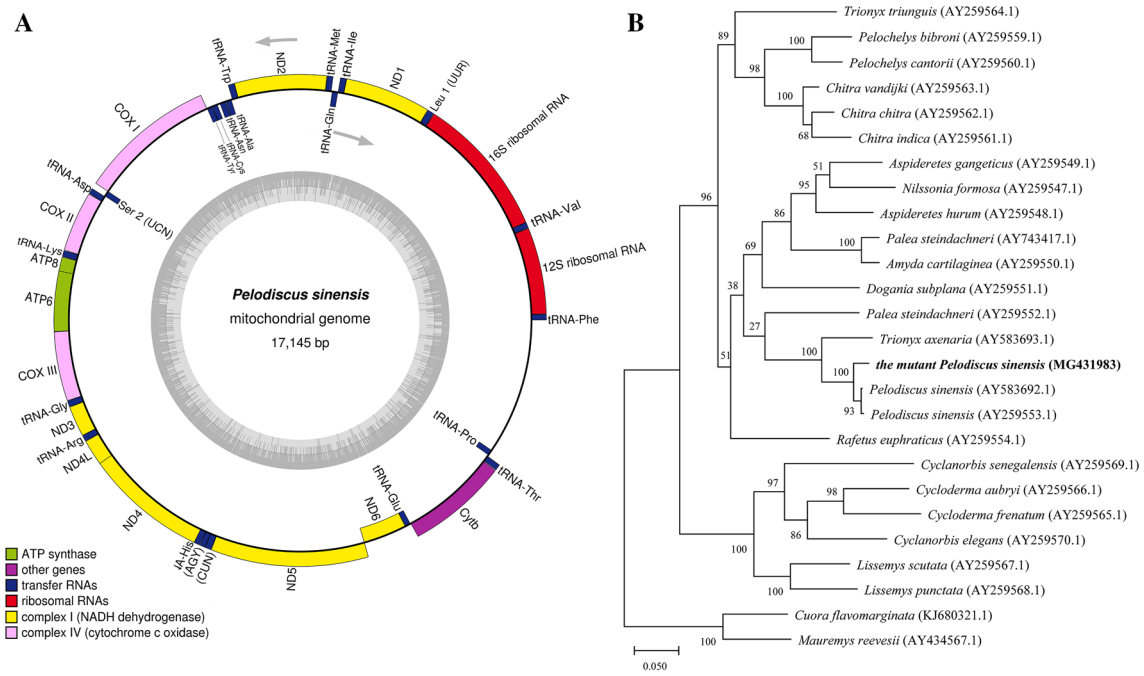

Fig. 1 **a** Structure of the mitochondrial genome of the mutant *Pelodiscus sinensis*. **b** ML tree based on the sequences of cytb genes from the mitochondrial genomes of 24 representative Trionychidae species and two relative outgroups. Numbers near each node denote the bootstrap supports for ML analysis

rRNA were 979 and 1604 bp respectively, with AT contents of 59.3 and 62.4% respectively. O_L was 31 bp long and located between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes and the AT content of O_L was 67.7%. CR was 1625 bp long and located between the $tRNA^{Pro}$ and $tRNA^{Phe}$ genes with an AT content of 63.5%.

All PCGs were found to start with ATG, except the COXI gene which began with GTG. In all 13 PCGs, four complete stop codons (TAG, AGA, TAA, and AGG) and one incomplete stop codon (T) were found (Table 1). The incomplete stop codon is presumably completed to become the stop codon TAA by polyadenylation (Ojala et al. 1981).

To investigate the phylogenetic relationships between the mutant *P. sinensis* and other Trionychidae species, we constructed a phylogenetic tree using maximum likelihood (ML) analysis. The analysis was based on the nucleotide sequences of cytb genes from 24 turtles in the Trionychidae family; two turtles from the order Testudinidae was set as outgroups. The phylogenetic tree shows that Trionychidae species form two main branches, one composed of the genera *Cyclanorbis* and *Lissemys*, and the other branch containing other soft-shell turtles. *Trionyx axenaria* is the closest modern relative of *P. sinensis* (Fig. 1b).

The present results are in favor of the conservation of this VU species and provide fundamental molecular data for further genetic study and evolutionary analyses.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors are responsible for the content and writing of the paper.

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