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Characterization of the complete mitochondrial genome of the Rock pigeon, *Columba livia* (Columbiformes: Columbidae)

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ABSTRACT. The rock pigeon (*Columba livia*), or Rock dove, is a member of the bird family Columbidae. We mapped the complete mitochondrial genome of the Rock pigeon. The mitochondrial genome of this species is a circular molecule of 17,229 bp in length, encoding a standard set of 13 protein-coding genes, two ribosomal RNA genes, and 22 transfer RNA genes, plus a putative control region, demonstrating a structure very similar to that of other birds. As found in other vertebrates, most of these genes are coded on the H-strand, except for NADH dehydrogenase subunit 6 (*nad6*) and eight tRNA genes (Gln, Ala, Asn, Cys, Tyr, Ser(UCN), Pro, Glu). The AT skew and GC skew of the whole genome, protein-coding genes, tRNA, rRNA, and the control region were calculated for the complete mitochondrial genomes of 30 avian species, representing 29 orders. All protein-coding genes initiated with ATG, except for *cox1* and *nad5*, which began with GTG. One extra nucleotide ‘C’ was present in NADH dehydrogenase subunit 3 (*nad3*). All tRNA gene sequences have the potential to fold into typical cloverleaf secondary structures. Within the control region, conserved sequences were identified in three domains. Although the conserved blocks, such as ETAS1, ETAS2, CSB1, CSB1-like, and boxes C, D, E, and F, are readily identifiable in the *C. livia* control region, the typical origin of H-strand replication (O_H), CSB2 and CSB3 could not be detected. These results provide basic information for phylogenetic analyses of birds, especially Columbiformes species.

Key words: Mitochondrial genome; Rock pigeon; *Columba livia*; Columbiformes

INTRODUCTION

There are around 10,000 living species of birds, making them the most numerous tetrapod vertebrates. All modern birds are classified within the subclass Neornithes, which has two subdivisions: the Palaeognathae, containing mostly flightless birds, like ostriches, and the very diverse Neognathae, containing all other birds (Livezey and Zusi, 2007). Columbiformes is one of the most easily recognized avian orders worldwide and is traditionally subdivided into two families, the Columbidae and the Raphidae (Pereira et al., 2007). The Columbidae currently include over 300 species of pigeons and doves. The Rock pigeon (*Columba livia*), or Rock dove, is a member of the family Columbidae. This species includes the domestic pigeon, the feral pigeon, and the wild Rock pigeon. Wild Rock pigeons are pale grey, with two black bars on each wing; although domestic and feral pigeons are very variable in color and pattern. The Rock pigeon has a restricted natural resident range in western and southern Europe, north Africa, and south Asia. This species has been domesticated for several thousand years, giving rise to the domestic pigeon. Many domestic birds have escaped or have been released over the years, and have given rise to the feral pigeon. Feral pigeons are found in large numbers in cities and towns all over the world.

Genome-scale approaches have played an important role in the inference of evolutionary relationships among diverse organisms (Rokas et al., 2003). Compared with the nuclear genome, the mitochondrial genome has several intrinsic characteristics (small genome size, fast substitution rate and maternal inheritance mode), which render the mitogenome as one of the most suitable markers for phylogenetic (Wang et al., 2008) and population genetics studies, such as analysis of gene flow, hybridization and introgression (Moore, 1995).

Typical metazoan mitochondrial genomes are double-stranded circular molecules, ranging in size from approximately 15 to 20 kb and containing 37 genes: 13 protein-coding genes, 22 transfer RNA genes (tRNAs) and two ribosomal RNA genes (rRNAs) (Boore, 1999). Additionally, one major control region is believed to control the initiation of replication and transcription of animal mitochondrial DNA (mtDNA) (Shadel and Clayton, 1997). To date, nearly 110 complete mtDNA sequences are available for 28 avian species (Table 1); however, no complete mitochondrial sequence has been published for members of the order Columbiformes, except for one sequence deposited in GenBank (NC_013244). Here, we present the complete mitochondrial genome of the Rock pigeon, *C. livia* (Columbiformes: Columbidae) and give a thorough description of its genome features in comparison to other avian species.

Table 1. List of avian species examined in this study, classified according to Howard and Moore (2003).

Order	Family	Species	Accession No.	Reference
Neognathae				
Anseriformes	Anatidae	*Branta canadensis*	NC_007011	Unpublished data[a]
Apodiformes	Apodidae	*Apus apus*	NC_008540	Unpublished data[b]
Caprimulgiformes	Aegothelidae	*Aegotheles cristatus*	NC_011718	(Pratt et al., 2009)
Charadriiformes	Haematopodidae	*Haematopus ater*	NC_003713	(Paton et al., 2002)
Ciconiiformes	Ciconiidae	*Ciconia ciconia*	NC_002197	Unpublished data[c]
Columbiformes	Columbidae	*Hemiphaga novaeseelandiae*	NC_013244	Unpublished data[d]
Coraciiformes	Coraciidae	*Eurystomus orientalis*	NC_011716	(Pratt et al., 2009)
Cuculiformes	Neomorphidae	*Geococcyx californianus*	NC_011711	(Pratt et al., 2009)
Falconiformes	Falconidae	*Falco peregrinus*	NC_000878	(Mindell et al., 1998)
Galliformes	Phasianidae	*Gallus gallus*	NC_007236	(Nishibori et al., 2005)
Gaviiformes	Gaviidae	*Gavia stellata*	NC_007007	(Slack et al., 2006)
Gruiformes	Rhynochetidae	*Rhynochetos jubatus*	NC_010091	(Morgan-Richards et al., 2008)
Passeriformes	Menuridae	*Menura novaehollandiae*	NC_007883	(Slack et al., 2007)
Pelecaniformes	Phaethontidae	*Phaethon rubricauda*	NC_007979	Unpublished data[e]
Phoenicopteriformes	Phoenicopteridae	*Phoenicopterus ruber*	NC_010089	(Morgan-Richards et al., 2008)
Piciformes	Ramphastidae	*Pteroglossus azara*	NC_008549	(Gibb et al., 2007)
Podicipediformes	Podicipedidae	*Tachybaptus novaehollandiae*	NC_010095	(Morgan-Richards et al., 2008)
Procellariiformes	Diomedeidae	*Thalassarche melanophrys*	NC_007172	(Slack et al., 2006)
Psittaciformes	Psittacidae	*Melopsittacus undulatus*	NC_009134	Unpublished data[f]
Rheiformes	Rheidae	*Rhea americana*	NC_000846	(Harlid et al., 1998)
Sphenisciformes	Spheniscidae	*Eudyptula minor*	NC_004538	(Slack et al., 2003)
Strigiformes	Strigidae	*Ninox novaeseelandiae*	NC_005932	(Harrison et al., 2004)
Struthioniformes	Struthionidae	*Struthio camelus*	NC_002785	(Haddrath and Baker, 2001)
Tinamiformes	Tinamidae	*Tinamus major*	NC_002781	(Haddrath and Baker, 2001)
Trochiliformes	Trochilidae	*Archilochus colubris*	NC_010094	(Morgan-Richards et al., 2008)
Trogoniformes	Trogonidae	*Trogon viridis*	NC_011714	(Pratt et al., 2009)
Palaeognathae				
Apterygiformes	Apterygidae	*Apteryx haastii*	NC_002782	(Haddrath and Baker, 2001)
Casuariiformes	Casuariidae	*Casuarius casuarius*	NC_002778	(Haddrath and Baker, 2001)
Dinornithiformes	Dinornithidae	*Dinornis giganteus*	NC_002672	(Cooper et al., 2001)

Unpublished data: [a]Snyder JC, Snider AR, Senecal AJ, Disantis EJ, et al.; [b]Slack KE, Delsuc F, McLenachan PA, Bartosch-Haerlid A, et al.; [c]Yamamoto Y; [d]Gibb GC, Goldberg J, Trewick SA, Powlesland RG, et al.; [e]Yamamoto Y, Kakizawa R and Yamagishi S; [f]Guan X, Samuels DC and Smith EJ.

MATERIAL AND METHODS

Sample collection and DNA extraction

An adult domestic male Rock pigeon was collected in Wuhu, Anhui Province, China. Total genomic DNA was extracted from the muscle tissue using standard phenol/chloroform methods (Sambrook and Russell, 2001).

PCR amplification and sequencing

Natural transfer of DNA from the mitochondria to the nucleus generates nuclear copies of mtDNA (NUMTs) and is an ongoing evolutionary process (Hazkani-Covo et al., 2010). To minimize the possibility of obtaining NUMTs, two long overlapping fragments

(~12 kb in length) were first amplified using the long and accurate-polymerase chain reaction (LA-PCR) kit (Takara, Dalian, China). The first LA-PCR primer set was LA16SF (5'-CCTACGTGATCTGAGTTCAGACCGGAGCAATCCAG-3') of Nishibori et al. (2001) and CytbR252 (5'-GATGCAGATGAAGAAGAATGAGGCGCCGTTTGC-3') designed based on the sequence of the cytochrome b gene (*cob*) from Cabot's tragopan. The second primer set was LACLMTF (5'-GAAGCATCATCTCCCACCTAGAATGAAAATTC-3') and LACLMTR (5'-CCCTCCACCAGCAGGATCAAAGAAGGTAGTGTT-3') based on certain sequences of *C. livia* using the above LA-PCR primer set (Table 1). The LA-PCR was conducted at 94°C for 1 min, followed by 35 cycles consisting of 10 s denaturation at 98°C and 13 min annealing and extension at 68°C, with a final extension step of 10 min at 72°C, in a DNA thermal cycler TC-3000 (Techne, Barloworld Scientific Ltd., UK). The amplified fragments with a size of approximately 12 kb that were obtained were used as the templates for amplification of the short overlapping fragments (1.1-1.5 kb in length) with 15 primer sets (Table 2). All 15 sets of primers were designed based on the conserved sequences of the known mitochondrial genome sequences in Galliformes or on the determined sequences of fragments of the mitogenome of *C. livia* in this study, which were aligned using CLUSTAL X v2.0.10 (Larkin et al., 2007). Each primer set amplified an mtDNA fragment containing an overlap of at least 100 bp with the adjacent amplified fragment at both termini. PCR was carried out in a volume of 25 µL containing 5-50 ng DNA template, 6.25 pmoL of each primer, 0.2 mM of each dNTP, 2 mM $MgCl_2$, and 0.625 U *DreamTaq*™ DNA polymerase (Fermentas, Burlington, Canada) or Ex *Taq*

Table 2. Primers used in amplifying and sequencing *Columba livia*.

Fragment	Primer name	Primer sequence (5'-3')	Primer size (bp)
1	CLMT1F	TATCACACCTCCCTACACCG	20
	CLMT1R	TCGTGATGGATACTTCTCGC	20
2	AVMT2F	AACCCATTATATGTATACGG	20
	AVMT2R	TTACTGCTGAGTACCCGTGG	20
3	AVMT3F	GCAAAAGACTTAGTCCTAACC	21
	AVMT3R	CTTTTGCGACAGAGACGGGTT	21
4	AVMT4F	AAGTCGTAACAAGGTAAGTGAC	22
	AVMT4R	CGCCCCAACCGAAAAATGTC	20
5	AVMT5F	AAGACGAGAAGACCCTGTGG	20
	CLMd5F	CGCAGTACCAATCCTAATC	19
	CLMT5R	CGGTTTGTTTCAGCGAGAGTT	21
6	AVMT6F	TAAGCACCCTGGCCATCACC	20
	CLMT6R	GGTTAGAAGGGTGAGGTTTG	20
7	AVMT7F	ACACAGACACGAAAAATCCT	20
	CLMT7R	AAGGTCTACAGAGGCTCCG	19
8	AVMT8F	CGCATAAATAACATAAGCTTC	21
	AVMT8R	GAAGCATTAAGTGGTTTGAT	20
9	AVMT9F	AAGCCTTCTCAGCAAAACGA	20
	AVMT9R	GCTTAGGTTCATGGTCAGGT	20
10	AVMT10F	ATGACATGCCCCAATTAAACC	21
	AVMT10R	GATGGCTTGTTTTCGGTTTCC	21
11	AVMT11F	CAAGCCTAGCCCCAACACCAG	21
	AVMT11R	ATGGGGTTAGTCAGTGTAGGC	21
12	ColMT12F	CTCATGGTTCAGATCACTTAC	21
	ColMT12R	GTTCCTCGTTGGGTTATTAG	20
13	AVMT13F	ACTACGAACGGACACACAGCCG	22
	AVMT13R	GAAGG CCAAA TTGAG CGGAT	20
14	AVMT14F	ATGACAAGGACGAGCTGAAG	20
	AVMT14R	ATTATTTTTAGTAGGGGGTG	20
15	AVMT15F	GCCAA CCTTC ATCTC ACCATAA	22
	CLMd15R	AGGGTTTGCAGGCGTGAAGT	20
	AVMT15R	CTTGTGCGTGGGTTGTCTCGGG	22

DNA polymerase (TaKaRa, Dalian, China). Amplification was conducted in a DNA thermal cycler TC-3000, as above. PCR cycles were as follows: one cycle of 4 min at 70°C, 4 cycles of 40 s at 94°C, 20 s at 52°C, and 2 min and 10 s at 72°C, followed by 36 cycles of 20 s at 94°C, 20 s at 50-55°C, and 2 min and 10 s at 72°C. The process was completed with a final extension step at 72°C for 10 min. The band with the right size was cut out and purified using an EZ Spin Column DNA Gel Extraction Kit (Bio Basic Inc.) and then cloned with pGEM®-T Easy Vector System II (Promega). Internal primers were applied to fragments 5 and 15 (Table 2). All distinct clones were sequenced on an ABI-PRISM 3730 sequencer using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the corresponding primer.

Gene identification and genome analyses

DNA sequences were analyzed using BioEdit 7.0.9.0 (Hall, 1999) and DAMBE 4.1.19 (Xia and Xie, 2001) softwares. Contig assembly was performed with the ContigExpress program (a component of Vector NTI Suite 6.0). The boundaries of protein-coding genes and rRNA genes were initially identified via DOGMA (Wyman et al., 2004) using the default setting, and refined by alignment with mitochondrial genomes of other avian species (Table 1). Composition skewness was calculated according to the formulas (AT skew = [A - T] / [A + T]; GC skew = [G - C] / [G + C]) (Perna and Kocher, 1995). Most tRNA genes were identified using tRNAscan-SE1.21 (Lowe and Eddy, 1997) under the 'cove only' search mode, with the vertebrate mitochondrial genetic code and 'mito/chloroplast' source. Some tRNA genes that were not found by the tRNA-SE1.21 were identified by proposed secondary structures and anti-codons (Shen et al., 2009). A gene map of the mitochondrial genome of *C. livia* was initially generated with OGDRAW (Lohse et al., 2007) and then modified manually.

RESULTS AND DISCUSSION

Genome organization and base composition

The complete sequence of the mtDNA of *C. livia* is 17,229 bp in length (Figure 1 and Table 3) and was deposited in GenBank with the accession No. (GU908131). The size of avian mtDNA ranges from 16,595 bp (*Ninox novaeseelandiae*) to 18,967 bp (*Thalassarche melanophrys*). Similar to the typical mitochondrial genomes of other vertebrates, the Rock pigeon mtDNA consists of 13 typical protein-coding genes, 22 tRNA genes, 2 rRNA genes (*srRNA* and *lrRNA*; small and large rRNA subunits, respectively) and one putative control region (D-loop) (Table 3). As found in other vertebrates, most of these genes are coded on the H-strand, except for one protein-coding gene (*nad6*) and eight tRNA genes ($tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, $tRNA^{Ser(UCN)}$, $tRNA^{Pro}$, $tRNA^{Glu}$).

The overall base composition of the H-strand is as follows: A (30.15%), T (23.98%), G (13.98%), C (31.90%); the A+T content of *C. livia* (54.13%) is similar to those of other birds (ranging from 52.46 to 57.56%, Table 4). As in most vertebrates, the overall base composition is skewed against guanine in the *C. livia* mitochondrial genome, which is due to a strong bias against the use of guanine at the third codon position (San Mauro et al., 2004).

The nucleotide composition of the *C. livia* mitogenome is slightly biased toward A+T nucleotides (54.13%, Table 4), which is a higher percentage than that of *P. azara* (52.46%),

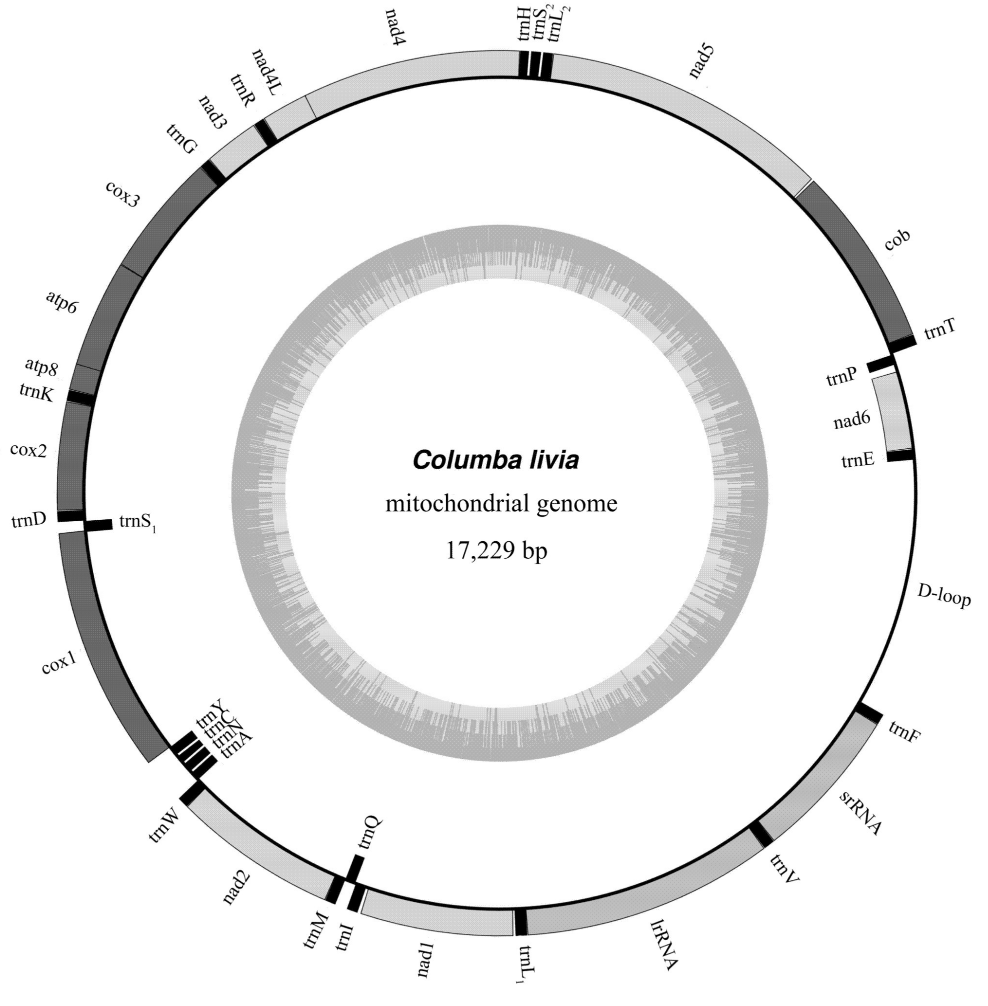

Figure 1. Gene map of the mitochondrial genome of *Columba livia*. Genes encoded on the heavy or light strands are shown outside and inside the circular gene map, respectively. The inner ring displays the GC content. Twenty-two tRNA genes are designated by single-letter amino acid codes. This figure was initially generated with OGDRAW and modified manually.

P. rubricauda (52.75%), *B. canadensis* (52.78%), *N. novaehollandiae* (52.8%), *R. americana* (53.06%), *C. ciconia* (53.67%), and *G. gallus* (53.99%), but lower than in the other 22 avian species, ranging from 54.17 to 57.56%. Within 13 protein-coding genes in the *C. livia* mitochondrial genome, the A+T composition is the highest in the *atp8* gene (55.95%), and the lowest in the *cob* gene (51.53%).

Table 3. Localization and features of genes in the mitochondrial genome of *Columba livia*.

Gene/region	Strand	Position		Size		Codon		Anticodon	Intergenic nucleotides[b]
		From	To	Nucleotide	Amino acids	Start	Stop[a]		
$tRNA^{Phe}$	H	1	69	69				GAA	0
srRNA	H	70	1,042	973					0
$tRNA^{Val}$	H	1,043	1,114	72				TAC	0
lrRNA	H	1,115	2,700	1586					0
$tRNA^{Leu(UUR)}$	H	2,701	2,774	74				TAA	11
nad1	H	2,786	3,751	966	324	ATG	AGA		17
$tRNA^{Ile}$	H	3,769	3,839	71				GAT	5
$tRNA^{Gln}$	L	3,845	3,915	71				TTG	-1
$tRNA^{Met}$	H	3,915	3,983	69				CAT	0
nad2	H	3,984	5,023	1040	346	ATG	TA-		0
$tRNA^{Trp}$	H	5,024	5,094	71				TCA	1
$tRNA^{Ala}$	L	5,096	5,164	69				TGC	2
$tRNA^{Asn}$	L	5,167	5,239	73				GTT	2
$tRNA^{Cys}$	L	5,242	5,308	67				GCA	-1
$tRNA^{Tyr}$	L	5,308	5,379	72				GTA	1
cox1	H	5,381	6,931	1551	516	GTG	AGG		-9
$tRNA^{Ser(UCN)}$	L	6,923	6,996	74				TGA	2
$tRNA^{Asp}$	H	6,999	7,067	69				GTC	2
cox2	H	7,070	7,753	684	227	ATG	TAA		1
$tRNA^{Lys}$	H	7,755	7,825	71				TTT	1
atp8	H	7,827	7,994	168	54	ATG	TAA		-10
atp6	H	7,985	8,668	684	227	ATG	TAA		-1
cox3	H	8,668	9,451	784	261	ATG	T-		0
$tRNA^{Gly}$	H	9,452	9,520	69				TCC	0
nad3	H	9,521	9,872	352		ATT	TAA		1
$tRNA^{Arg}$	H	9,874	9,942	69				TCG	1
nad4L	H	9,944	10,240	297	98	ATG	TAA		-7
nad4	H	10,234	11,611	1378	459	ATG	T-		0
$tRNA^{His}$	H	11,612	11,680	69				GTG	0
$tRNA^{Ser(AGY)}$	H	11,681	11,746	66				GCT	-1
$tRNA^{Leu(CUN)}$	H	11,746	11,817	72				TAG	0
nad5	H	11,818	13,632	1815	604	GTG	AGA		11
cob	H	13,644	14,786	1143	380	ATG	TAA		0
$tRNA^{Thr}$	H	14,787	14,855	69				TGT	6
$tRNA^{Pro}$	L	14,862	14,931	70				TGG	45
nad6	L	14,977	15,498	522	173	ATG	TAG		3
$tRNA^{Glu}$	L	15,502	15,572	71				TTC	0
D-loop	H	15,573	17,229	1657					0

[a] "-" Indicates termination codons completed via polyadenylation. [b] Negative values represent overlapping nucleotides.

The AT skew and GC skew were calculated for the complete mitogenomes of 30 avian species, representing 29 orders (Table 4). The AT skew for the *C. livia* mitochondrial genome is slightly positive (0.114), indicating the occurrence of more As than Ts. We found similar results in the other 29 avian species (0.068 to 0.180). In contrast, the GC skew in all 30 avian mitochondrial genomes was strongly negative (-0.346 to 0.438), meaning that there is a heavy bias toward Cs and against Gs. Furthermore, one extra nucleotide 'C' is present in *nad3* of *C. livia*, which is consistently observed in many other birds and some turtles and is thought not to be translated (Slack et al., 2003). The function of the extra "C" in *nad3* and its phylogenetic implications are still unclear.

Table 4. Composition and skewness in avian mitochondrial genomes.

Species	Size (bp)[a]	A%	C%	G%	T%	A+T%	AT skew	GC skew
Whole genome								
A. apus	17,037	31.37	31.52	13.19	23.92	55.29	0.135	-0.410
A. colubris	16,356	30.62	31.27	14.22	23.86	54.48	0.124	-0.375
A. cristatus	18,607	30.67	30.54	13.63	25.10	55.77	0.100	-0.383
A. haastii	16,980	30.91	29.48	13.33	26.24	57.15	0.082	-0.377
B. canadensis	16,760	30.18	32.07	15.14	22.60	52.78	0.144	-0.359
C. casuarius	16,756	30.47	29.73	14.45	25.25	55.72	0.094	-0.346
C. ciconia	17,347	30.54	31.98	14.35	23.13	53.67	0.138	-0.381
C. livia	**17,229**	**30.15**	**31.90**	**13.98**	**23.98**	**54.13**	**0.114**	**-0.391**
D. giganteus	17,070	30.67	31.25	13.93	24.15	54.82	0.119	-0.383
E. minor	17,611	30.96	31.84	13.53	23.67	54.63	0.133	-0.404
E. orientalis	17,774	30.26	31.74	13.82	24.18	54.44	0.112	-0.393
F. peregrinus	18,068	32.74	30.87	13.57	22.77	55.51	0.180	-0.389
G. californianus	17,091	32.34	31.70	12.40	23.55	55.89	0.157	-0.438
G. gallus	16,785	30.25	32.49	13.52	23.74	53.99	0.121	-0.412
G. stellata	17,573	30.36	31.43	14.39	23.81	54.17	0.121	-0.372
H. ater	16,791	31.59	31.12	13.67	23.62	55.21	0.144	-0.390
H. novaeseelandiae	17,264	30.97	31.89	13.21	23.92	54.89	0.128	-0.414
M. undulates	18,193	31.13	31.73	12.96	24.19	55.32	0.125	-0.420
M. novaehollandiae	17,839	30.11	30.36	14.45	25.08	55.19	0.091	-0.355
N. novaehollandiae	16,223	30.83	33.40	13.78	21.97	52.80	0.168	-0.416
P. azara	18,736	28.44	34.10	13.44	24.02	52.46	0.084	-0.435
P. rubber	17,446	31.54	32.03	13.49	22.93	54.47	0.158	-0.407
P. rubricauda	17,777	29.41	32.95	14.29	23.34	52.75	0.115	-0.395
R. american a	16,714	28.69	32.20	14.74	24.37	53.06	0.081	-0.372
R. jubatus	16,937	30.32	30.36	14.15	25.17	55.49	0.093	-0.364
S. camelus	16,595	30.47	30.40	14.21	24.92	55.39	0.100	-0.363
T. major	16,701	30.48	29.88	13.05	26.56	57.04	0.069	-0.392
T. melanophrys	18,967	31.45	30.53	13.64	24.38	55.83	0.127	-0.382
T. novaehollandiae	18,002	31.75	32.06	13.12	23.08	54.83	0.158	-0.419
T. viridis	17,751	30.73	28.82	13.62	26.83	57.56	0.068	-0.358
Protein-coding genes								
A. apus	11,388	29.00	32.74	13.03	25.22	54.22	0.070	-0.431
A. colubris	11,394	28.59	32.23	13.83	25.35	53.94	0.060	-0.400
A. cristatus	11,388	28.77	32.04	13.57	25.62	54.39	0.058	-0.405
A. haastii	11,385	29.16	30.41	13.10	27.33	56.49	0.032	-0.400
B. canadensis	11,412	27.97	33.04	15.12	23.86	51.83	0.079	-0.372
C. casuarius	11,394	28.23	30.65	14.12	27.01	55.24	0.022	-0.369
C. ciconia	11,400	28.42	33.89	13.63	24.05	52.47	0.083	-0.426
C. livia	**11,388**	**27.96**	**32.98**	**13.68**	**25.38**	**53.34**	**0.048**	**-0.414**
D. giganteus	11,397	28.28	32.27	13.85	25.60	53.88	0.050	-0.400
E. minor	11,400	29.16	33.83	13.01	24.00	53.16	0.097	-0.444
E. orientalis	11,397	28.20	33.25	13.73	24.80	53.00	0.064	-0.416
F. peregrinus	11,406	29.49	32.52	13.19	24.80	54.29	0.086	-0.423
G. californianus	11,403	30.23	32.29	12.56	24.92	55.15	0.096	-0.440
G. gallus	11,397	28.31	33.58	13.37	24.74	53.05	0.067	0.430
G. stellata	11,400	28.48	33.49	13.78	24.25	52.73	0.080	-0.417
H. ater	11,400	29.75	32.15	13.14	24.96	54.71	0.088	-0.420
H. novaeseelandiae	11,391	28.99	32.67	13.21	25.13	54.12	0.071	-0.424
M. undulates	11,400	29.89	33.60	12.26	24.25	54.14	0.104	-0.465
M. novaehollandiae	11,394	27.92	32.25	14.40	25.43	53.35	0.047	-0.383
N. novaehollandiae	11,406	29.03	33.72	13.49	23.73	52.76	0.100	-0.429
R. jubatus	11,379	26.09	36.34	13.60	23.97	50.06	0.042	-0.455
P. azara	11,400	29.18	32.92	13.49	24.41	53.59	0.089	-0.419
P. rubber	11,397	27.12	34.61	14.64	23.64	50.76	0.069	-0.405
P. rubricauda	11,403	26.01	33.78	14.57	25.64	51.65	0.007	-0.397
R. americana	11,397	28.53	31.70	13.64	26.14	54.66	0.044	-0.398
S. camelus	11,400	28.01	31.21	14.24	26.54	54.55	0.027	-0.373
T. major	11,382	28.40	30.50	12.91	28.20	56.60	0.004	-0.405
T. melanophrys	11,406	29.14	31.45	13.56	25.85	54.99	0.060	-0.397
T. novaehollandiae	11,388	30.30	33.42	12.78	23.50	53.80	0.126	-0.447
T. viridis	11,406	29.07	29.38	13.46	28.09	57.16	0.017	-0.372

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Table 4. Continued.

Species	Size (bp)	A%	C%	G%	T%	A+T%	AT skew	GC skew
tRNA								
A. apus	1,548	29.78	21.58	21.32	27.33	57.11	0.043	-0.006
A. colubris	1,551	30.37	20.95	21.41	27.08	57.45	0.057	0.011
A. cristatus	1,539	29.82	20.86	21.25	28.07	57.89	0.030	0.009
A. haastii	1,551	33.27	24.31	16.57	25.85	59.12	0.126	-0.189
B. canadensis	1,540	30.65	21.56	20.78	27.01	57.66	0.063	-0.018
C. casuarius	1,539	30.86	21.25	19.56	28.33	59.19	0.043	-0.041
C. ciconia	1,550	29.81	21.35	21.42	27.42	57.23	0.042	0.002
C. livia	**1,547**	**28.70**	**21.78**	**22.24**	**27.28**	**55.98**	**0.025**	**0.010**
D. giganteus	1,552	30.22	20.94	21.07	27.77	57.99	0.042	0.003
E. minor	1,548	29.20	21.77	21.96	27.07	56.27	0.038	0.004
E. orientalis	1,550	29.23	21.61	21.74	27.42	56.65	0.032	0.003
F. peregrinus	1,563	29.17	21.63	21.69	27.51	56.69	0.029	0.001
G. californianus	1,542	30.80	21.34	20.75	27.11	57.91	0.064	-0.014
G. gallus	1,543	30.14	21.84	20.67	27.35	57.49	0.049	-0.028
G. stellata	1,554	29.79	21.75	21.43	27.03	56.82	0.049	-0.007
H. ater	1,549	30.08	20.92	20.79	28.21	58.30	0.032	-0.003
H. novaeseelandiae	1,549	29.70	20.98	21.37	27.95	57.65	0.030	0.009
M. undulates	1,541	30.24	21.35	20.44	27.97	58.21	0.039	-0.022
M. novaehollandiae	1,548	29.72	20.93	21.45	27.91	57.62	0.031	0.012
N. novaehollandiae	1,557	29.42	22.41	21.71	26.46	55.88	0.053	-0.016
P. azara	1,546	29.50	21.54	21.73	27.23	56.73	0.040	0.004
P. rubber	1,551	29.72	21.79	21.28	27.21	56.93	0.044	-0.012
P. rubricauda	1,543	28.78	22.81	21.97	26.44	55.22	0.042	-0.019
R. american a	1,538	29.91	20.42	21.46	28.22	58.13	0.029	0.025
R. jubatus	1,555	29.65	21.29	21.74	27.33	56.98	0.040	0.010
S. camelus	1,538	30.82	20.22	20.81	28.15	58.97	0.045	0.014
T. major	1,537	30.45	20.04	20.82	28.69	59.14	0.030	0.019
T. melanophrys	1,757	30.17	20.38	21.29	28.17	58.34	0.034	0.022
T. novaehollandiae	1,532	29.63	21.48	21.67	27.22	56.85	0.042	0.004
T. viridis	1,555	30.42	20.51	20.96	28.10	58.52	0.040	0.011
rRNA								
A. apus	2,572	33.90	27.10	18.47	20.53	54.43	0.246	-0.189
A. colubris	2,553	33.37	26.48	19.31	20.84	54.21	0.231	-0.157
A. cristatus	2,561	32.02	27.22	19.25	21.28	53.30	0.202	-0.172
A. haastii	2,638	34.72	25.21	17.78	22.29	57.01	0.218	-0.173
B. canadensis	2,591	33.08	26.24	20.46	20.22	53.30	0.241	-0.124
C. casuarius	2,559	34.54	25.21	18.80	21.45	56.00	0.234	-0.146
C. ciconia	2,576	33.27	27.91	18.94	19.88	53.14	0.252	-0.191
C. livia	**2,559**	**32.75**	**26.26**	**19.42**	**21.57**	**54.32**	**0.206**	**-0.150**
D. giganteus	2,567	34.75	27.04	17.96	20.26	55.01	0.263	-0.202
E. minor	2,583	32.91	27.84	19.09	20.17	53.08	0.240	-0.186
E. orientalis	2,562	32.90	27.40	19.59	21.10	53.01	0.219	-0.166
F. peregrinus	2,578	33.67	27.97	18.89	18.47	53.14	0.292	-0.194
G. californianus	2,572	33.75	26.56	18.04	21.66	55.40	0.218	-0.191
G. gallus	2,598	33.06	28.37	18.17	20.40	53.46	0.237	-0.219
G. stellata	2,588	33.04	27.47	19.32	20.17	53.21	0.242	-0.174
H. ater	2,552	33.03	26.61	19.47	20.89	53.92	0.225	-0.155
H. novaeseelandiae	2,575	33.94	27.34	18.14	20.58	54.52	0.245	-0.202
M. undulates	2,549	33.58	30.29	18.12	18.01	51.59	0.302	-0.251
M. novaehollandiae	2,575	32.35	25.48	20.31	21.86	54.21	0.194	-0.113
N. novaehollandiae	2,557	32.93	29.25	19.63	18.19	51.11	0.288	-0.197
P. azara	2,556	32.08	29.93	18.97	19.01	51.10	0.256	-0.224
P. rubber	2,567	33.27	26.76	19.05	20.92	54.19	0.228	-0.168
P. rubricauda	2,581	33.05	29.99	18.09	18.87	51.92	0.273	-0.248
R. american a	2,546	32.88	26.75	19.17	21.21	54.08	0.216	-0.165
R. jubatus	2,553	31.84	25.85	19.98	22.33	54.17	0.176	-0.128
S. camelus	2,546	34.33	26.28	18.07	21.33	55.66	0.234	-0.185
T. major	2,575	34.06	26.06	18.02	21.86	55.92	0.218	-0.182
T. melanophrys	2,573	33.15	26.16	19.43	21.26	54.41	0.219	-0.148
T. novaehollandiae	2,563	34.14	28.25	18.14	19.47	53.61	0.274	-0.218
T. viridis	2,600	33.00	25.50	19.00	22.50	55.50	0.189	-0.146

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Table 4. Continued.

Species	Size (bp)	A%	C%	G%	T%	A+T%	AT skew	GC skew
Control region								
A. apus	21,470	34.49	24.56	11.90	29.05	63.54	0.086	-0.347
A. colubris	787	29.35	26.18	15.25	29.10	58.45	0.004	-0.264
A. cristatus	3,054	31.57	25.87	11.72	30.71	62.28	0.014	-0.376
A. haastii	1,351	25.54	25.91	12.66	35.90	61.44	-0.169	-0.344
B. canadensis	1,194	29.65	31.49	13.48	25.38	55.03	0.078	-0.400
C. casuarius	1,138	28.21	26.98	15.20	29.61	57.82	-0.024	-0.279
C. ciconia	1,779	31.08	22.60	17.93	28.39	59.47	0.045	-0.115
C. livia	**1,657**	**30.84**	**28.49**	**13.28**	**27.40**	**58.24**	**0.059**	**-0.364**
D. giganteus	1508	30.11	27.45	14.32	28.12	58.22	0.034	-0.314
E. minor	2,040	31.13	23.04	13.24	32.60	63.73	-0.023	-0.270
E. orientalis	2,190	30.78	25.89	11.60	31.74	62.51	-0.015	-0.381
F. peregrinus	2,460	39.88	22.48	14.02	23.25	63.13	0.263	-0.232
G. californianus	1,169	28.74	28.49	11.89	30.88	59.62	-0.036	-0.411
G. gallus	1,232	26.46	26.62	13.47	33.44	59.90	-0.117	-0.328
G. stellata	1,984	29.89	21.57	15.98	32.56	62.45	-0.043	-0.149
H. ater	1,240	32.02	25.97	14.68	27.34	59.35	0.079	-0.278
H. novaeseelandiae	1,690	30.41	29.88	11.78	27.87	58.28	0.044	-0.434
M. undulates	2,685	26.48	23.35	14.23	35.94	62.42	-0.152	-0.243
M. novaehollandiae	2,280	31.32	24.91	11.36	32.41	63.73	-0.017	-0.374
N. novaehollandiae	662	29.46	30.82	14.80	24.92	54.38	0.083	-0.351
P. azara	3,224	27.57	27.14	12.44	32.85	60.42	-0.087	-0.371
P. rubber	1,878	34.40	30.24	11.24	24.07	58.47	0.177	-0.458
P. rubricauda	2,211	29.76	25.33	11.85	33.06	62.82	-0.053	-0.363
R. american a	1,171	30.06	27.50	14.52	27.92	57.98	0.037	-0.309
R. jubatus	1,414	29.84	21.64	15.49	33.03	62.87	-0.051	-0.166
S. camelus	1,034	28.82	26.50	14.31	30.37	59.19	-0.026	-0.299
T. major	1,102	29.67	25.50	12.07	32.76	62.43	-0.049	-0.357
T. melanophrys	2,629	31.95	26.63	13.35	28.07	60.02	0.065	-0.332
T. novaehollandiae	2,437	28.60	25.44	14.53	31.43	60.03	-0.047	-0.273
T. viridis	2,128	29.56	25.89	11.94	32.61	62.17	-0.049	-0.369

[a]The control region of *A. colubris* and *N. novaehollandiae* are incomplete.

Protein-coding genes

The total length of the 13 protein-coding genes in *C. livia* mtDNA is 11,388 bp, accounting for 66.1% of the complete mitochondrial genome (Table 4). The length of the 13 protein-coding genes found in avian species varies from 11,379 (*Rhynochetos jubatus*) to 11,412 bp (*Branta canadensis*). The 13 protein-coding genes found in the *C. livia* mtDNA are similar in length to most other avian species. The longest protein-coding gene of *C. livia* mtDNA is the *nad*5 gene (1815 bp), whereas the shortest is the *atp*8 gene (168 bp; Table 5).

Analysis of the base composition at each codon position of the concatenated 13 protein-coding genes of *C. livia* mitochondrial genome shows that each codon position has a different AT/GC bias (Table 6). The first and third codon positions of *C. livia* are biased toward A and C, while the second codon positons are biased toward T and C. The AT composition at the first codon position is 49.1%. The values of the second and third codon positions are 58.5 and 52.5%, respectively (Table 6). As with most avian species (except for *A. haastii*, *T. major* and *T. viridis*), the second codon position has the highest AT composition (Table 6). The *nad6* gene of *C. livia* mitogenome has strong skews of T *vs* A (-0.587), and G *vs* C (0.602), while the *nad1* gene has a slight skew of T *vs* A (-0.016), and a strong skew of C *vs* G (0.452). All other 11 protein-coding genes of the *C. livia* mitochondrial genome have a slight skew of A *vs* T (0.005 to 0.151), and a strong skew of C *vs* G (GC skew = -0.328 to -0.757; Table 5).

Table 5. Base composition for protein-coding genes found in the mitochondrial genome of *Columba livia*.

Gene	Length (bp)	Proportion of nucleotides (%)					AT skew	GC skew
		A	C	G	T	A+T		
nad1	966	25.98	34.27	12.94	26.81	52.80	-0.016	-0.452
nad2	1041	31.70	34.20	10.18	23.92	55.62	0.140	-0.541
cox1	1551	26.11	31.98	16.18	25.73	51.84	0.007	-0.328
cox2	684	30.41	30.85	14.62	24.12	54.53	0.115	-0.357
atp8	168	32.14	38.69	5.36	23.81	55.95	0.149	-0.757
atp6	684	28.65	35.09	9.80	26.46	55.12	0.040	-0.563
cox3	786	26.97	33.21	15.01	24.81	51.78	0.042	-0.377
nad3	351	27.07	33.90	12.25	26.78	53.85	0.005	-0.469
nad4L	297	27.61	33.33	14.48	24.58	52.19	0.058	-0.394
nad4	1380	30.51	35.72	10.72	23.04	53.55	0.139	-0.538
nad5	1815	31.13	34.49	11.40	22.98	54.10	0.151	-0.503
cob	1143	26.95	35.87	12.60	24.58	51.53	0.046	-0.480
nad6	522	10.92	9.39	37.74	41.95	52.87	-0.587	0.602
Mean	876	27.40	32.38	14.10	26.12	53.52		

Table 6. Summary of base composition at each codon position of the concatenated 13 protein-coding genes in avian mitochondrial genomes.

Species	1st codon position					2nd codon position					3rd codon position				
	A%	T%	G%	C%	AT%	A%	T%	G%	C%	AT%	A%	T%	G%	C%	AT%
A. apus	28.5	21.2	21.9	28.4	49.7	18.3	39.7	12.8	29.2	58.0	40.2	14.8	4.3	40.7	55.0
A. colubris	28.0	21.9	22.1	28.0	49.9	18.5	39.8	12.6	29.0	58.3	39.2	14.4	6.8	39.6	53.6
A. cristatus	28.7	20.9	22.1	28.3	49.6	18.3	39.8	12.7	29.2	58.1	39.3	16.2	5.9	38.6	55.5
A. haastii	28.6	22.3	21.4	27.6	50.9	18.3	40.5	12.8	28.4	58.8	40.6	19.2	5.0	35.2	59.8
B. canadensis	27.3	20.3	23.8	28.7	47.6	18.2	40.4	12.8	28.6	58.6	38.4	11.0	8.8	41.8	49.4
C. casuarius	27.9	21.4	22.6	28.1	49.3	18.2	40.3	12.8	28.6	58.5	38.6	19.3	7.0	35.2	57.9
C. ciconia	28.4	19.6	22.3	29.7	48.0	18.3	39.4	12.8	29.4	57.7	38.5	13.1	5.8	42.6	51.6
C. livia	**28.5**	**20.6**	**22.2**	**28.7**	**49.1**	**18.4**	**40.1**	**12.7**	**28.9**	**58.5**	**37.0**	**15.5**	**6.2**	**41.4**	**52.5**
D. giganteus	28.1	21.0	22.1	28.8	49.1	18.2	40.2	12.8	28.8	58.4	38.6	15.6	6.6	39.2	54.2
E. minor	29.5	20.4	21.4	28.7	49.9	18.4	39.2	12.8	29.6	57.6	39.6	12.4	4.8	43.2	52.0
E. orientalis	28.5	20.3	21.9	29.3	48.8	18.5	39.9	12.7	28.8	58.4	37.6	14.1	6.6	41.6	51.7
F. peregrinus	29.5	20.7	21.4	28.4	50.2	18.4	39.5	12.8	29.4	57.9	40.7	14.3	5.3	39.7	55.0
G. californianus	30.2	20.9	21.0	27.9	51.1	18.5	39.7	12.5	29.3	58.2	42.0	14.2	4.1	39.7	56.2
G. gallus	28.8	20.8	21.7	28.6	49.6	18.1	39.9	12.7	29.3	58.0	38.0	13.5	5.7	42.8	51.5
G. stellata	28.5	20.0	22.1	29.4	48.5	18.6	39.9	12.7	28.9	58.5	38.3	12.9	6.6	42.2	51.2
H. ater	29.4	21.2	21.4	27.9	50.6	18.7	39.9	12.8	28.7	58.6	41.1	13.8	5.3	39.8	54.9
H. novaeseelandiae	28.5	20.8	22.0	28.8	49.3	18.5	40.0	12.8	28.7	58.5	40.0	14.6	4.9	40.6	54.6
M. undulates	29.4	20.8	20.8	28.9	50.2	18.6	39.4	12.4	29.6	58.0	41.6	12.5	3.6	42.3	54.1
M. novaehollandiae	27.5	20.5	23.5	28.4	48.0	18.2	40.2	12.9	28.6	58.4	38.0	15.5	6.8	39.7	53.5
N. novaehollandiae	28.9	19.8	21.5	29.8	48.7	18.3	39.0	12.7	30.1	57.3	40.0	12.4	6.3	41.4	52.4
P. azara	28.3	20.8	21.7	29.2	49.1	18.2	39.8	12.7	29.3	58.0	31.7	11.3	6.4	50.6	43.0
P. rubber	28.5	20.6	22.2	28.7	49.1	18.4	39.9	12.8	28.9	58.3	40.6	12.7	5.5	41.2	53.3
P. rubricauda	27.7	19.8	22.2	30.2	47.5	18.1	39.5	13.0	29.4	57.6	35.6	11.5	8.6	44.2	47.1
R. americana	27.0	21.0	22.5	29.4	48.0	18.0	40.0	12.9	29.0	58.0	33.0	15.8	8.2	42.9	48.8
R. jubatus	28.7	20.8	22.4	28.1	49.5	18.5	39.8	12.6	29.1	58.3	38.4	17.8	5.9	37.9	56.2
S. camelus	27.8	21.3	22.6	28.2	49.1	18.3	40.3	12.7	28.7	58.6	37.9	17.9	7.4	36.7	55.8
T. major	29.5	22.7	20.7	27.0	52.2	18.3	40.1	12.8	28.8	58.4	37.3	21.8	5.2	35.7	59.1
T. melanophrys	28.7	21.2	22.0	28.0	49.9	18.4	40.0	12.9	28.7	58.4	40.3	16.3	5.8	37.6	56.6
T. novaehollandiae	29.4	20.4	21.2	28.9	49.8	18.6	39.5	12.6	29.3	58.1	42.9	10.5	4.6	42.1	53.4
T. viridis	28.7	22.8	21.9	26.6	51.5	18.7	40.1	12.5	28.7	58.8	39.8	21.4	5.9	32.8	61.2

There are four reading frame overlaps within the mitochondrial genome of *C. livia* (*cox1* and $tRNA^{Ser(UCN)}$ share nine nucleotides; *atp8* and *atp6* share 10 nucleotides; *nad4L* and *nad4* share seven nucleotides; *atp6* and *cox3* share one nucleotide). Other overlaps are shown in Table 3.

Twelve genes (with the exception of *nad6*) of the 13 typical protein-coding genes (*nad1-6* and *4L*, *cox1-3*, *atp6* and *atp8*, *cob*) are encoded on the heavy strand. All protein-coding genes initiate with ATG, except for *cox1* and *nad5*, which begins with GTG. Five types of stop codons are used by the coding genes, including TAA for *nad3*, *cox2*, *atp8*, *atp6*, *nad4L*, and *cob*; AGA for *nad1* and *nad5*; TAG for *nad6*; AGG for *cox1*, and incomplete stop codon T- or TA- for *cox3*, *nad2* and *nad4*, respectively. The use of an incomplete stop codon T, a common mechanism for stopping protein translations, was also observed in other avian species.

The pattern of codon usage in the *C. livia* mtDNA was also studied (Table 7). There are 3785 codons for all the 13 protein-coding genes, after stop codons are excluded. The most frequently used amino acid was Leu (17.89%), followed by Thr (9.30%), Ala (7.67%), Ile (7.59%), and Ser (7.43%).

Table 7. Codon usage of 13 protein-coding genes in the mitochondrial genome of *Columba livia*.

Amino acid	Codon	Number	Frequency (%)	Amino acid	Codon	Number	Frequency (%)
Phe	TTT	47	1.24	Tyr	TAT	28	0.74
	TTC	166	4.37		TAC	86	2.27
Leu	TTA	65	1.71	Stop	TAA	9	0.24
	TTG	21	0.55		TAG	1	0.03
	CTT	76	2.00	His	CAT	22	0.58
	CTC	187	4.93		CAC	86	2.27
	CTA	299	7.88	Gln	CAA	79	2.08
	CTG	30	0.79		CAG	13	0.34
Ile	ATT	86	2.27	Asn	AAT	24	0.63
	ATC	202	5.32		AAC	105	2.77
Met	ATA	132	3.48	Lys	AAA	74	1.95
	ATG	40	1.05		AAG	9	0.24
Val	GTT	40	1.05	Asp	GAT	21	0.55
	GTC	53	1.40		GAC	43	1.13
	GTA	56	1.48	Glu	GAA	79	2.08
	GTG	22	0.58		GAG	18	0.47
Ser	TCT	34	0.90	Cys	TGT	7	0.18
	TCC	107	2.82		TGC	19	0.50
	TCA	78	2.05	Trp	TGA	93	2.45
	TCG	8	0.21		TGG	12	0.32
Pro	CCT	45	1.19	Arg	CGT	9	0.24
	CCC	82	2.16		CGC	16	0.42
	CCA	93	2.45		CGA	42	1.11
	CCG	5	0.13		CGG	6	0.16
Thr	ACT	58	1.53	Ser	AGT	13	0.34
	ACC	157	4.14		AGC	42	1.11
	ACA	130	3.42	Stop	AGA	2	0.05
	ACG	8	0.21		AGG	1	0.03
Ala	GCT	50	1.32	Gly	GGT	27	0.71
	GCC	139	3.66		GGC	80	2.11
	GCA	93	2.45		GGA	80	2.11
	GCG	9	0.24		GGG	32	0.84

Non-coding regions

The non-coding regions in the *C. livia* mitochondrial genome include a control region (D-loop) and a few spaces (Table 3). The control region (1657 bp) is located between *tRNA*^{Glu} and *tRNA*^{Phe} genes. The length of the control region of avian species varies between 1034 (*Struthio camelus*) and 3224 bp (*Pteroglossus azara*), ranging in AT content from 55.03 (*B. canadensis*) to 63.73% (*E. minor* and *M. novaehollandiae*; Table 4).

Based on the distribution of the variable nucleotide positions and differential frequencies of the nucleotides, the mitochondrial control region is divided into three domains (Brown et al., 1986; Saccone et al., 1991; Randi and Lucchini, 1998). The nucleotide composition of the *C. livia* control region was A = 30.84, T = 27.40, C = 28.49, and G = 13.28% (Table 4), with a bias against G, which is usual for the mtDNA sense strand of vertebrates (Wolstenholme, 1992). Domain I (ETAS, extended termination-associated sequences) contains part A (nt 1-146 in Figure 2) and part B (nt 147-398 in Figure 2). In part A, ETAS1 and ETAS2 are found at positions 71-126 and 108-146 nt, respectively, and overlapped one another by 19 bp, with 63.3 and 58.7% similarity to the consensus mammalian ETAS1 and ETAS2, respectively (Sbisa et al., 1997). In part B (nt 147-398), a CSB1-like block (5'-TAGAACTTAATGATACC TTAGACATA-3') has 63.0% similarity to the conserved sequence block (CSB1) in domain III (Figure 2). Four conserved sequence boxes in the central domain II (nt 399-897) were identified as boxes C, D, E, and F (Figure 2). In domain III (nt 898-1657), there is a poly (T) sequence (nt 1123-1131), which is located just a few nucleotides downstream from the putative CSB1 (nt 983-1007; Figure 2). Although the conserved blocks, such as ETAS1, ETAS2, CSB1, CSB1-like, and boxes C, D, E, and F, are readily identifiable in the *C. livia* control region, the typical origin of H-strand replication (O_H), CSB2 and CSB3 could not be detected (Walberg and Clayton, 1981). The bidirectional light- and heavy-strand transcription promoters (LSP/HSP) (L'Abbé et al., 1991) are found in *C. livia* (Figure 2). Furthermore, there are 27 tandem repeats (CAAA) at the end of the *C. livia* control region.

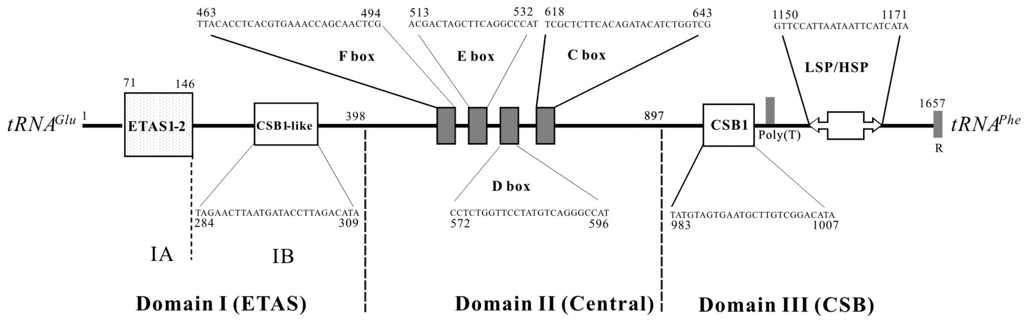

Figure 2. Schematic representation of the organization of the *Columba livia* control region. ETAS = extended termination-associated sequences; F through C boxes = conserved sequence boxes in the central domain; CSB = conserved sequence block; CSB-like = a sequence similar to the CSB; LSP = light-strand transcription promoter; HSP = heavy-strand transcription promoter; R = tandem repeats.

Ribosomal and transfer RNA genes

The Rock pigeon mitochondrial genome has two rRNA subunits (*srRNA* and *lrRNA*), as in other vertebrates. The *srRNA* gene is located between $tRNA^{Phe}$ and $tRNA^{Val}$ genes, and the *lrRNA* gene is located between $tRNA^{Val}$ and $tRNA^{Leu(UUR)}$ genes. The lengths of *srRNA* and *lrRNA* are 973 and 1586 bp. The A+T content of the *rRNA* is 54.32%, which is within the range observed for other avian species (Table 4).

Twenty-two tRNA genes were observed in the Rock pigeon mitochondrial genome. The tRNA genes are interspersed in the genome, ranging in size from 66 ($tRNA^{Ser(AGY)}$) to

74 (*tRNA*^{Trp}) nucleotides (Table 3). *tRNA*^{Cys} and *tRNA*$^{Ser(AGY)}$, which were not found by the tRNAscan-SE, were identified by comparison with *G. gallus* counterparts. All tRNA gene sequences have the potential to fold into typical cloverleaf secondary structures (Figure 3). The DHU and TΨC arms contain two and five nucleotide pairs, respectively.

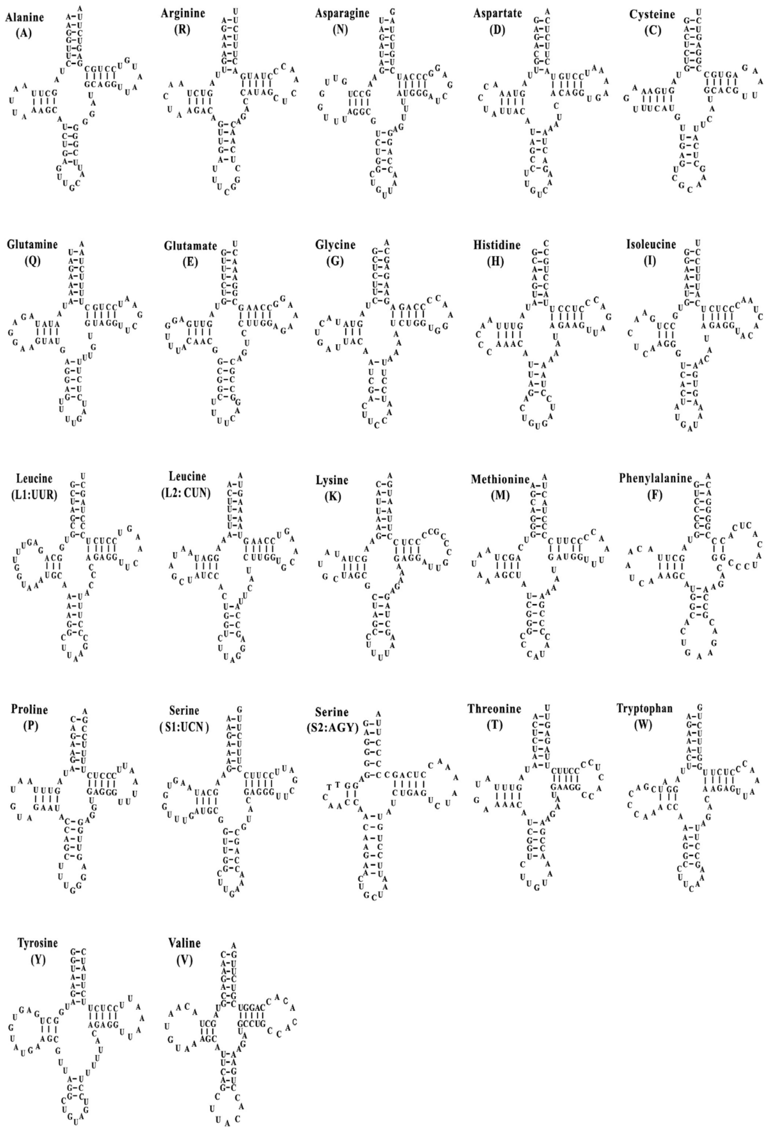

Figure 3. Inferred secondary structures of 22 tRNAs found in the *Columba livia* mitochondrial genome.

This is the first complete nucleotide sequence described for the mitochondrial genome of the Rock pigeon. We also detailed the genome organization and codon usage of *C. livia* mitochondrial DNA. These results provide basic information for phylogenetic analyses among the birds, and especially Columbiformes species.

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