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The mitochondrial genome of the Saunders's gull *Chroicocephalus saundersi* (Charadriiformes: Laridae) and a higher phylogeny of shorebirds (Charadriiformes)

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ABSTRACT

The complete mitogenome of *Chroicocephalus saundersi* was characterized and compared with the 6 published Charadriiformes mitogenomes. The mitogenome of *C. saundersi* is a closed circular molecule 16,739 bp in size, and contains 37 genes and a control region. The AT and GC skews are positive and negative, respectively, and in agreement with those of the other Charadriiformes mitogenomes. The mitogenome of *C. saundersi* contains 3 start codons (ATG, GTG, and ATT), 4 stop codons (TAA, TAG, AGG, and AGA), and an incomplete stop codon (T–) in 13 PCGs. A codon usage analysis of all available Charadriiformes mitogenomes showed that the ATG (78%) and TAA (50.5%) were the most common start codon and stop codon, respectively. An unusual start codon, ATT, is commonly found in the *ND3*s of Charadriiformes mitogenomes, whereas the more common start codons, ATC and ATA, are rarely found. In all the Laridae species, one extra cytosine was inserted at position 174 in *ND3*. The control region of *C. saundersi* is 1180-bp long, with a nucleotide composition of 30.2% A, 28.6% T, 27.3% C, and 14.0% G. Variable numbers of tandem repeats (VNTRs) with nine copies of the 10 bp repeat sequence (AACAACAAAC) are found within the CSB domain of the control region. The ML/BI analyses, based on the amino acids of the 13 mitochondrial PCGs, strongly support the monophyly of the order Charadriiformes, with the suborder Lari considered sister to the Scolopaci, which is in turn a sister group to the suborder Charadrii.

1. Introduction

Animal mitogenomes are generally 15–20 kb double-stranded circular molecules (Boore, 1999) that consist of 13 protein-coding genes (PCGs), two ribosomal RNAs (12S rRNA and 16S rRNA), 22 transfer RNAs (tRNAs), and a non-coding control region (CR) containing the signal genes required for replication and transcription (Wolstenholme, 1992; Ruokonen and Kvist, 2002). The genome-level characteristics of mitogenomes include their base composition, codon usage and gene order arrangement, tRNA and rRNA gene secondary structures, and modes of replication and transcription (Boore, 1999; Lei et al., 2010). Such information has been utilized to investigate higher-level phylogeny among taxa, in evolutionary studies, and for developing conservation management programs for endangered species.

Despite recent rapid increases in the available information on bird mitogenomes, the complete mitogenomes of only 6 species in the order Charadriiformes (shorebirds) have been characterized (Table 1). Of these, four mitogenomes were sequenced from species in the suborder Lari (Laridae and Alcidae), with the other two being sequenced from species in the suborders Scolopaci (Scolopacidae) and Charadrii (Haematopodidae) (Paton et al., 2002; Yamamoto et al., 2005; Slack et al., 2007; Yang et al., 2012, 2014).

Chroicocephalus saundersi (Saunders's gull), a species of gull in the suborder Lari (Laridae), is known to breed in the coastal regions of eastern China and the west coast of South Korea (Bird Life International, 2012; Kwon et al., 2012). This seabird has a small, declining population estimated to include only 21,000 to 22,000 individuals. Its rate of population decline is likely to increase in the next 10 years, because of land reclamation projects carried out on tidal flats and the disturbance of breeding colonies (Bird Life International, 2012). Thus, *C. saundersi* has been rated as "vulnerable" by the IUCN, and classified as an endangered species by the Korean Ministry of the Environment.

Abbreviations: dNTP, deoxyribonucleoside triphosphate; aa, amino acid(s); rRNA, ribosomal RNA; tRNA, transfer RNA; PCGs, Protein coding genes; ND1, NADH dehydrogenase subunit 1; ND2, NADH dehydrogenase subunit 2; COI, cytochrome *c* oxidase subunit I; COII, cytochrome *c* oxidase subunit II; ATP8, ATP synthase F0 subunit 8; ATP6, ATP synthase F0 subunit 6; COIII, cytochrome *c* oxidase subunit III; ND3, NADH dehydrogenase subunit 3; ND4L, NADH dehydrogenase subunit 4L; ND4, NADH dehydrogenase subunit 4; ND5, NADH dehydrogenase subunit 5; CytB, cytochrome *b*; ND6, NADH dehydrogenase subunit 6.

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Table 1
Mitogenomes of the order Charadriiformes used in this study.

Suborder	Family	species	Size (bp)	Accession no.	Reference
Lari	Laridae	*Chroicocephalus saundersi*	16,739	KJ_631624[a]	–
		Chroicocephalus brunnicephalus	16,769	NC_018548	Yang et al. (2012)
		Ichthyaetus relictus	16,586	NC_023777	Yang et al. (2014)
		Larus dominicanus	16,701	NC_007006	Slack et al. (2007)
	Alcidae	*Synthliboramphus antiquus*	16,730	NC_007978	Yamamoto et al. (2005)
Scolopaci	Scolopacidae	*Arenaria interpres*	16,725	AY_074885	Paton et al. (2002)
Charadrii	Haematopodidae	*Haematopus ater*	16,791	NC_003713	Paton et al. (2002)

[a] The mitogenome sequence (KJ_631624) was obtained in this study.

In this study, we characterized the complete mitogenome of *C. saundersi* and analyzed the nucleotide compositions and codon usage biases of the Charadriiformes mitogenomes, in order to understand the evolutionary forces shaping them and infer the higher phylogeny of the order Charadriiformes.

2. Materials and methods

2.1. Sample collection and DNA extraction

C. saundersi breeds in tidal flats and saltmarshes in Song-do (Incheon Metropolitan City) on the west coast of South Korea. Using a 10 mL syringe, we extracted a 5 mL blood sample from a young *C. saundersi* individual within a week of its hatching at a breeding colony. The blood sample was preserved in tubes containing heparin and stored at −40 °C. Total genomic DNA was extracted from the blood sample using the DNeasy® Blood & Tissue Kit (Qiagen, Valencia, CA, USA), according to the manufacturer-supplied protocols.

2.2. Primer design, PCR amplification, and DNA sequencing

The primers were designed based on sequence-conserved regions, which were identified using multiple alignments of the complete mitogenomes of *Chroicocephalus brunnicephalus*, *Larus dominicanus*, *Ichthyaetus relictus*, *Synthliboramphus antiquus*, *Arenaria interpres*, and *Haematopus ater*, which are available in GenBank (Paton et al., 2002; Yamamoto et al., 2005; Slack et al., 2007; Yang et al., 2012, 2014) (Table 1). PCR amplification using the primer pairs newly designed for this study (Suppl. Table 1) was conducted in a final reaction volume of 25 μL, which contained 10 mM Tris–HCl (pH 8.4), 50 mM KCl, 4 mM $MgCl_2$, 200 mM of each dNTP, 50 pmol each primer, 2 U ExTaq polymerase, and 1 μL of the DNA sample, under the following conditions: 94 °C for 5 min (initial denaturation); then 94 °C for 1 min (denaturation),

Table 2
Gene organization of the *Chroicocephalus saundersi* mitogenome.

Gene	Start position	Stop position	Length (bp)	Anticodon	Start codon	Stop codon	Strand
tRNAPhetRNAPhetRNAPhe	1	72	72	GAA			+
12S rRNA	73	1037	965				+
tRNAValtRNAValtRNAVal	1038	1109	72	TAC			+
16S rRNA	1110	2711	1602				+
tRNALeu(UUR)tRNALeu(UUR)tRNALeu(UUR)	2712	2785	74	TAA			+
ND1	2789	3766	978		ATG	AGA	+
tRNAIletRNAIletRNAIle	3775	3846	72	GAT			+
tRNAGlntRNAGlntRNAGln	3856	3926	71	TTG			−
tRNAMettRNAMettRNAMet	3926	3994	69	CAT			+
ND2	3995	5035	1041		ATG	TAG	+
tRNATrptRNATrptRNATrp	5034	5107	74	TCA			+
tRNAAlatRNAAlatRNAAla	5109	5177	69	TGC			−
tRNAAsntRNAAsntRNAAsn	5180	5252	73	GTT			−
tRNACystRNACystRNACys	5255	5321	67	GCA			−
tRNATyrtRNATyr	5321	5391	71	GTA			−
COI	5393	6943	1551		GTG	AGG	+
tRNASer(UCN)tRNASer(UCN)	6935	7008	74	TGA			−
tRNAAsptRNAAsp	7011	7079	69	GTC			+
COII	7081	7764	684		ATG	TAA	+
tRNALystRNALys	7766	7835	70	TTT			+
ATP8	7837	8004	168		ATG	TAA	+
ATP6	7995	8678	684		ATG	TAA	+
COIII	8678	9461	784		ATG	T–	+
tRNAGlytRNAGly	9462	9530	69	TCC			+
ND3	9531	9882	351		ATT	TAA	+
tRNAArgtRNAArg	9887	9955	69	TCG			+
ND4L	9957	10,253	297		ATG	TAA	+
ND4	10,247	11,624	1378		ATG	T–	+
tRNAHistRNAHis	11,625	11,693	69	GTG			+
tRNASer(AGY)tRNASer(AGY)	11,694	11,759	66	GCT			+
tRNALeu(CUN)	11,759	11,829	71	TAG			+
ND5	11,830	13,644	1815		GTG	AGA	+
CytB	13,664	14,806	1143		ATG	TAA	+
tRNAThr	14,811	14,880	70	TGT			+
tRNAPro	14,888	14,957	70	TGG			−
ND6	14,963	15,484	522		ATG	TAG	−
tRNAGlu	15,488	15,559	72	TTC			−
D-loop	15,560	16,739	1180				+