# Analysis of the complete mitogenome of Oriental turtle dove (*Streptopelia orientalis*) and implications for species divergence

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## ARTICLE INFO

## ABSTRACT

The Oriental turtle dove, *Streptopelia orientalis* (Columbiformes: Columbidae) is an extremely widespread species. We investigate and analyze its complete mitochondrial genome. The complete mtDNA genome, a circular DNA molecule of 17,130bp, contains of 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a control region. Its base composition is A 29.7%, T 23.9%, G 14.1%, C 32.3%. Similar to other birds and turtles, the Oriental turtle dove has an extra base "C" at position 174 of gene *Nd3*. The most frequently codon usage is CUA (7.11%), followed by AUC (5.52%), CUC (4.97%) and UUC (4.36%). Genetic distance analyses indicate that the difference between *Macropygia phasianella* vs *Spilopelia senegalensis* is lower than that *Spilopelia chinensis* vs *S. orientalis* based on both the standard barcoding gene *Cox1* and *Cytb*. Genetic distances coincide with morphological, behavioral and phylogenetic analyses that suggest the assignment of *chinensis* and *senegalensis* to the genus *Spilopelia*, following the latest taxonomic system of the IUCN.

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## 1. Introduction

The Oriental turtle dove, *Streptopelia orientalis* (Columbiformes: Columbidae), is an extremely widespread species that inhabits a remarkable diversity of habitats (MacKinnon et al., 2000). Until now, only partial fragments of its mitochondrial DNA (*Atp8*, *Atp6*, *Cox1*, *Nd2*, *Cytb*, and D-loop), ribosomes (18S rRNA) and nuclear DNA data (*Drd4*, β-fibrinogenintron 7) have been determined (Abe et al., 2011; Chikuni et al., 1995; Johnson, 2004; Johnson and Clayton, 2000; Johnson et al., 2001; Okumura et al., 2005; Saitoh et al., 2014; Seki, 2006). With more than 300 member species of pigeons and doves, only six extant columbids (*Columba livia*, *Geotrygon violacea*, *Hemiphaga novaeseelandiae*, *Leptotila verreauxi*, *Spilopelia chinensis*, *Zenaida auriculata*) and one extinct species (*Ectopiste migratorius*) have complete mitogenomes (Khan and Arif, 2013). Therefore, more mitochondrial genomes within Columbidae should be determined.

Controversy remains as to the generic placement of the spotted dove *S. chinensis*, a species related to *S. orientalis* (MacKinnon et al., 2000; Zheng, 2011; Cheke, 2005; BirdLife International, 2014). Many authorities have assigned *orientalis*

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and *chinensis* to the genus *Streptopelia* (MacKinnon et al., 2000; Zheng, 2011). In contrast, a study based on molecular data and vocalizations reported that *S. chinensis* and the laughing dove, *Spilopelia senegalensis*, stood out (Johnson et al., 2001). Later, Cheke (2005) proposed that *chinensis* and *senegalensis* be united in the genus *Stigmatopelia* yet the IUCN has used genus *Spilopelia*, which has priority over *Stigmatopelia* (BirdLife International, 2014). Below, we apply the latest taxonomic system of IUCN (BirdLife International, 2014).

Herein, we determine the complete mitochondrial genome of *S. orientalis*, analyze nucleotide composition, and codon usage. We also report DNA barcoding using partial fragments of mitochondrial cytochrome oxidase subunit 1 (*Cox1*) and cytochrome b (*Cytb*) genes and calculate the levels of sequence divergence among several related species, including *S. chinensis*, *S. orientalis*, *S. senegalensis* and the brown cuckoo-dove *Macropygia phasianella* to gain a perspective on the generic placement of the species *S. chinensis* and *S. senegalensis*.

## 2. Material and methods

### 2.1. Ethics statement

The sample collection was strictly conducted under national ethical guidelines (Regulations for Administration of Affairs Concerning Experimental Animals, China, 1988) for animal husbandry and humane treatment.

### 2.2. Sample collection

A specimen of *S. orientalis* was collected from Ji'an city, Jiangxi Province, China and the muscle sample was associated with the voucher specimen (zjbj2) at vouched in the Jinggangshan University.

### 2.3. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissue using a standard phenolchloroform extraction protocol (Sambrook et al., 1989). Primers combinations were designed with the reference sequences of previously published mitogenomes of the related species *C. livia* (NC_013978) and *S. chinensis* (KP636801). Eleven overlapping PCR products, ranging from 936 to 2944 bp in length, were amplified to cover the entire mitogenome. Primers used for the generation of PCR products and the sizes of the fragments were given in Table S1.

PCR protocol was set as follows: an initial denaturation at 94 °C for 4 min; 35 cycles of amplification as the main procedure: a denaturation at 95 °C for 30s, an annealing at 48–60 °C for 45 s, an elongation at 72 °C for 45 s–180 s, and a final extension at 72 °C for 7min. The product was stored at 4 °C. The amplification was performed in 25 μl reaction volumes with 2.5 μl of 10 × PCR buffer ($Mg^{2+}$ Free; TaKaRa Biotech, Dalian, China), 2.5 μl $MgCl_2$ (25 mM), 1.0–4.0 μl deoxyribonucleoside triphosphates (dNTP, 2.5 mM), 1.0 μl each primer (10 μM), 0.2 μl [EX] Taq polymerase (5 U/μl), and approximately 200 ng total genomic DNA as template. All PCR products were examined through electrophoresing on a 1.0% agarose gel and purified with the DNA Agarose Gel Extraction Kit (Omega, Norcross, Georgia, USA) and then directly sequenced with the primer walking method in an ABI 3730xl sequencer.

### 2.4. Sequence analysis

All de novo sequences were assembled and edited using the software package SeqMan (DNASTAR 7.1.0) (Swindell and Plasterer, 1997). The boundaries of protein-coding genes were predicted by homologous sequences of other doves using the software Mega 6.0 (Tamura et al., 2013). The transfer RNA (tRNA) genes were identified using tRNA-scan SE 1.21 (Lowe and Eddy, 1997). The tRNA-Lys and tRNA-Ser(AGY) genes, which were not found using tRNA-scan SE, were identified by the observation of already proposed secondary rRNA structures (Kumazawa and Nishida, 1993) and investigation of the anticodons. The maps of the mitochondrial genome and control region of *S. orientalis* (Swindell and Plasterer, 1997) was drawn by SeqBuilder (DNASTAR 7.1.0).

### 2.5. Phylogenetic analyses

We downloaded the *Cyt b* and *Cox1* sequences for 35 columbids species using in Johnson et al. (2001) and combined the corresponding sequences, which determined in this study. For each gene, neighbor-joining method was performed using Mega 6.0 (Tamura et al., 2013) with K2P model.

### 2.6. Genetic distance analysis

To investigate the generic placement of *S. orientalis*, we retrieved 11 *Cox1* sequences representing the species *S. senegalensis* (HQ168037, HQ168038, HQ168039), *S. chinensis* (JF498901, JQ176290, KP636801), *S. orientalis* (AB843774, AB843775) and *M. phasianella* (JQ175307, JQ175308, JQ175309). We also evaluated *Cytb* sequences for *S. chinensis* (KP636801), *S.*