

The complete mitochondrial genome of Lesser Sand-Plover *Charadrius mongolus atrifrons* and its phylogenetic position

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ABSTRACT

The Lesser Sand-Plover (*Charadrius mongolus atrifrons*) is a small shorebird in Charadriiformes. Here we assembled the complete mitochondrial genome of *C. m. atrifrons* (Aves: Charadriiformes) which is 16,919 bp in length and consisting of 13 protein-coding (PCGs), 2 ribosomal RNA, 22 transfer RNA and 1 control region. The overall A + T content of was 55.5%. The Maximum Likelihood (ML) tree based on the 12 concatenated mitochondrial protein-coding genes (except ND6 gene) placed *C. m. atrifrons* in a clade with *C. alexandrinus* but separate from *C. vociferus*.

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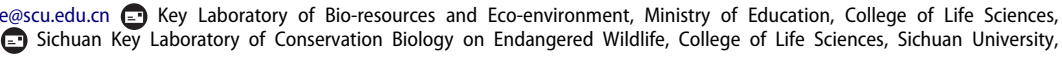
Charadriiformes is a species-rich clade of shorebirds, which is currently represented by 13 family-level taxa in China (Zheng 2017). The phylogenetic relationships within shorebirds have been well studied based on nuclear and/or short mitochondrial sequences (Barth et al. 2013; Remedios et al. 2015; Chen et al. 2018), but the phylogenetic placement of many species has not been addressed with complete mitochondrial genomes. To date, correctly identified complete mitochondrial genomes have been published for only six species of Charadriidae, which hampers studies of the ecology, evolutionary biology and population genetics of these shorebirds. The Lesser Sand-Plover (*Charadrius mongolus atrifrons*) is a long-distance migratory bird in Charadriiformes, whose mitochondrial DNA have not been well-studied. In this study, we sequenced and analyzed the complete mitochondrial genome of *C. m. atrifrons*, providing a basis for future studies of population genetics, evolution, phylogeny and conservation genetics.

Muscle tissue of a wild *C. m. atrifrons* collected from an airport protection facility was gathered from Nagri Kunsha Airport (80.055442E, 32.100026N) in July 2020. The specimen was stored in the Natural Museum of Sichuan University with a voucher number of QZKK063 (Curator name: Jianghong Ran; Email: rjhong-01@163.com). The total genomic DNA was extracted using the M5 HiPer Universal DNA Mini Kit following the manufacturer's instructions. The mitogenome of *C. m. atrifrons* were generated by amplification of overlapping Polymerase Chain Reaction (PCR) fragments. The thirteen fragments were amplified using the universal primers (Amer

et al. 2013) following the instructions of 2× Rapid Taq Master Mix (Vazyme Biotech Co., Ltd). Sequences obtained were aligned and annotated using the software SeqMan (DNASTar, Inc.) and MITOS Web Server (Bernt et al. 2013), respectively.

The circular mitochondrial genome of *C. m. atrifrons* (GenBank accession number MW298528) was 16,919 bp in length, consisting of 13 protein-coding (PCGs), 2 ribosomal RNA (rRNA), 22 transfer RNA (tRNA) and 1 control region, which is consistent with other vertebrate mitogenomes (Xie et al. 2016). All the genes of *C. m. atrifrons* are encoded on the H-strand with the exception of ND6 and 8 tRNAs (tRNA^{Gln}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys}, tRNA^{Tyr}, tRNA^{Ser(UCN)}, tRNA^{Pro} and tRNA^{Glu}). The overall nucleotide base composition was 32.0% A, 30.5% C, 13.9% G, and 23.5% T. The overall A + T content of 55.5%. The AT skew was calculated as 0.15.

To determine the phylogenetic position of *C. m. atrifrons*, PhyloSuite (Zhang et al. 2020) was used to construct a maximum-likelihood (ML) phylogenetic tree of seven species (*Pluvialis apricaria*, *P. fulva*, *Vanellus cinereus*, *V. vanellus*, *Charadrius vociferus*, *C. alexandrinus*, and *C. m. atrifrons*). *Tringa nebularia* (Zhang et al. 2019) was used as an outgroup (Figure 1). GTR + F + I + G4 was selected as the substitution model according to the Bayesian Information Criterion (BIC) test based on Modeltest. The Maximum Likelihood phylogeny placed the members of Charadriidae in four major groups. *C. m. atrifrons* was placed with *C. alexandrinus* but distant from *C. vociferus*. This pattern is consistent with the results of

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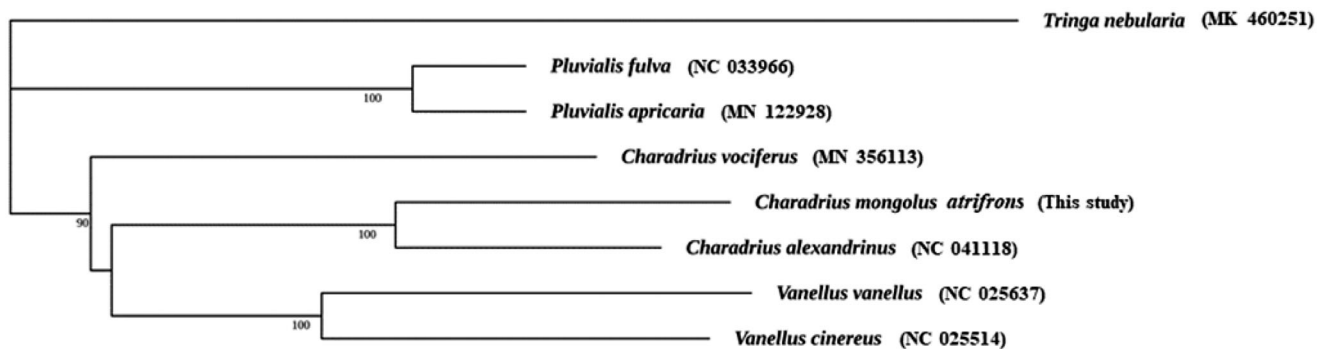


Figure 1. Phylogenetic tree of *Charadrius mongolus atrifrons* based on the maximum likelihood (ML) analysis of 12 concatenated mitochondrial protein-coding genes. *Tringa nebularia* was set as outgroup. The bootstrap values are based on 1000 replicates and shown at the nodes.

Barth et al. (2013) and Remedios et al. (2015) who found that the genus *Charadrius* is paraphyletic due to the inclusion of *Vanellus*. Indeed, it has been suggested that some species of *Charadrius*, including *C. m. atrifrons*, should be placed in a different genus *Anarhynchus* (Sangster et al. 2016). This pattern was inconsistent with the placement of *Charadrius* plovers and *Vanellus* lapwings in separate genera (Dickinson & Remsen, 2013). In conclusion, our study described the complete mitogenome of *C. m. atrifrons* and investigated its phylogenetic position, which may benefit future studies of ecology, evolutionary biology and population genetics in shorebirds.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, under the accession MW298528. The associated BioProject, SRA, and Bio-

Sample numbers are PRJNA741831, SRP326305 and SAMN19908120 respectively.

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