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The complete mitochondrial genome of *Accipiter virgatus* and evolutionary history of the pseudo-control regions in Falconiformes

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ABSTRACT

The complete mitochondrial genome sequence of *Accipiter virgatus* was determined. This mt-genome was 17,952 bp in length and consisted of 22 tRNA genes, 13 protein-coding genes, 2 rRNA genes, one control region (CR) and one pseudo-control region (CCR). Phylogenetic analyses of 14,644 bp of mitochondrial DNA (12 protein-coding genes, 2 rRNAs and 22 tRNAs) revealed the phylogenetic position of Cathartidae (*Cathartes aura*) was more closer to Ciconiidae (storks) than Accipitridae. To investigate the divergence times of the CCRs in Falconiformes, detailed analyses of the noncoding regions (CR and CCR) were performed. We found the recently reported novel gene order in Falconiformes had multiple independent origins and hence cannot be used to infer phylogenetic lineages. Indeed, the molecular clock suggested the CCR in Falconidae emerged about 65.4 million years (Mya), while that in Pandionidae–Accipitridae clade emerged about 19.16 Mya. The intra-genomic homology between the noncoding regions was detected in *Spilornis cheela*, which supporting the duplication hypothesis. Furthermore, the structure of CCR should be featured by a region containing tandem repeats as two definitely separated clusters of tandem repeats were found. The findings presented here should be considered in future phylogenetic and evolutionary studies targeting the pseudo-control regions of all Falconiformes species.

1. Introduction

The besra *Accipiter virgatus* Temminck, 1822, was enlisted as a class II protected species in China. This species is distributed in Bangladesh, Cambodia and China, and so on (Mees, 1984; IUCN, 2014). It belongs to the genus *Accipiter*, family Accipitridae with no doubt (Breman et al., 2013). Several studies based on molecular data revealed the phylogenetic relationships within

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Falconiformes were contentious (Breman et al., 2013; Mahmood et al., 2014). One example is the controversial relationship between Ciconiiformes (i.e., storks and ibises) and Cathartidae (*Cathartes aura*) (Ericson et al., 2006; Livezey and Zusi, 2007; Hackett et al., 2008). Fortunately, multiple sequences had sufficient information and were better than single gene for phylogenetic analysis of animals (Pacheco et al., 2011; Zhang and Zhang, 2013). This tactics should serve to further resolve the phylogenetic relationships within the Falconiformes when more mt-genomes are available.

On the other hand, the publication of the first complete avian mitochondrial genome (*Gallus gallus*) revealed the mitochondrial gene order of that differs from the arrangement prevalent in typical vertebrate gene order (Desjardins and Morais, 1990). The mitochondrial gene order of chicken (*G. gallus*) is shared by most avian lineages and appears to represent the typical avian gene order. However, Mindell et al. (1998) discovered a new mitochondrial gene rearrangement in *Falco peregrinus*, which one more noncoding sequence was found between the $tRNA^{Glu}$ and $tRNA^{Phe}$ genes except the original control region between $tRNA^{Thr}$ and $tRNA^{Pro}$. Subsequently, similar rearrangement was detected in representatives of Passeriformes, Procellariiformes, Cuculiformes, Piciformes and Psittaciformes (Mindell et al., 1998; Eberhard et al., 2001; Abbott et al., 2005). Recombination has been invoked as a mechanism to explain the mitochondrial rearrangement (Tang et al., 2000; Rokas et al., 2003). Moreover, several studies suggested the duplicate control regions could either evolve independently or evolve in concert (Arndt and Smith, 1998; Shao et al., 2005). In order to consist with earlier studies (Haring et al., 1999; Väli, 2002; Cadahía et al., 2009), we maintained to define the term CCR as the pseudo-control region located downstream of the functional CR.

Though many aspects of CR in modern birds were well studied, we still know little about CCR. Regarding raptors, or birds of prey, there has been some good progress in recent years. For example, the intra-genomic similarity between CR and CCR have been detected in two lineages of Falconiformes species (Pandionidae and Aquilinae), and tandem duplication followed by deletion of redundant copies of genes have been suggested the most plausible pattern for the generation of duplicate control regions in mt-genomes (Gibb et al., 2007; Cadahía et al., 2009). Further, the CCR has been used as a molecular marker for phylogenetic studies (Väli, 2002). However, all these findings pointed out that it is necessary to collect broad taxonomic samples, and more details about CCR should be studied.

Here we sequenced the complete mitochondrial genome of *A. virgatus*. Together with other published 17 complete mt-genome sequences (Table 1), we attempted to address the phylogenetic position of *C. aura*. We also investigated whether the similarity between the noncoding regions continues to exist in other Falconiformes species. Furthermore, to further our understanding of the evolution of CCR in Falconiformes, we performed intra- and intergenomic sequence comparisons and a molecular clock analysis.

2. Materials and methods

2.1. DNA sample

Muscle of a dead besra was collected from the Huangtianba flower market, Qingyang District, Chengdu City, Sichuan Province. Total DNA was extracted using standard phenol/chloroform methods (Sambrook and Russell, 2002).

2.2. PCR amplification and sequencing

We designed 12 pairs of primers (Appendix 1) to amplify the mt-genome of *A. virgatus*. PCR (polymerase chain reaction) was performed following the protocols described by He et al. (2009). PCR cycling was carried out on a PTC-100 thermal cycler (BioRad, Hercules, CA). The PCR products were purified using the DNA agarose gel extraction kit (Omega Bio-Tek, Norcross, GA). Purified products were sequenced on an ABI PRISM 3730 DNA sequencer by Invitrogen Biotechnologies Company (Carlsbad, CA).

2.3. Sequence analysis

DNA sequences were assembled and analyzed using the software MEGA 5.0 program (Tamura et al., 2011). The locations of protein-coding and rRNA genes were determined by comparison with corresponding known sequences from two other species: *Accipiter gentilis* and *Buteo buteo*. The tRNA genes were identified with tRNAscan-SE v.1.21 (Lowe and Eddy, 1997). However, three tRNA genes which were not found with tRNAscan-SE, identified by proposed secondary structures (Kumazawa and Nishida, 1993) and anti-codons.

For mitochondrial pseudo-control region comparisons, following sequences from GenBank were used: *Aquila chrysaetos*, *Aquila heliaca*, *Aquila nipalensis*, *Aquila clanga*, *Aquila pomarina* (AF435093–AF435099 and AF487438–AF487453), *A. gentilis* (FJ627047), *Hieraaetus fasciatus* (FJ627048), *Haliaeetus albicilla* (AY034150).

2.4. Phylogenetic analysis

To further address the phylogenetic relationships within the Falconiformes, 12 heavy-strand protein-coding genes, 2 rRNA genes and 22 tRNAs were automatically extracted from complete genome sequences according to GenBank annotations by using GenScalpel (Yin et al., 2012). All these sequences were aligned according to Pozzi et al. (2014). We deleted gaps,