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Complete Mitochondrial Genome of Blue-Throated Bee-eater *Merops viridis* (Coraciiformes: Meropidae) with its Taxonomic Consideration

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ZH, FT and DK designed the study and wrote the arctile. TF analyzed the sequence data and submitted to the Genbank.

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Merops viridis, Mitochondrial genome, Cytb, Gene rearrangement, Taxonomic consideration.

ABSTRACT

Blue-throated Bee-eater *Merops viridis* (Coraciiformes: Meropidae) is a bird species of family Meropidae with a very large distribution. The complete mitochondrial genome of Blue-throated Bee-eater *M. viridis* was determined. The mitogenome is a circular DNA molecule of 18,295 bp and comprises of 13 protein-coding genes, 22 tRNA genes, two rRNA genes and two control regions CR and CCR, which was first reported in the order Coraciiformes. The overall A+T content for the mitogenome is 52%, and the GC and AT skews are -0.400 and 0.108. Unlike to many other birds, no extra base is inserted at certain position relative to ND3. Interestingly, a 484bp repeated sequence appears in both CR and CCR. Genetic distance shows that the differences between *M. v. viridis* and *M. v. americanus* is higher than that *M. v. viridis* vs *M. leschenaulti* based on Cyt b gene. Morphology, geographical distribution and genetic data suggest the elevation of *M. v. americanus* to a full species Rufous-crowned Bee-eater *M. americanus*, supporting the latest taxonomic arrangement by IUCN.

INTRODUCTION

The bee-eaters (family Meropidae) comprise a group of 25 species of birds with brightly colored, morphologically homogeneous (Dickinson, 2003). They harbor unique feeding habits, and predominantly eat flying insects, especially bees and wasps (Fry *et al*., 1992). Blue-throated Bee-eater (*Merops viridis*) is a species of bird in the family Meropidae. The species has a very large distribution ranging from southern China and South-East Asia to the Greater Sundas and the Philippines (MacKinnon *et al*., 2000; Collar, 2011). Its natural habitat is subtropical or tropical mangrove forests. The species is characterized by a high ornamental value and listed in species checklists of conservation, economic and scientific values.

So far, only partial fragments of its mitochondrial DNA (mtDNA) (Cytb, 12S, ND1 and ND2) were determined. Hitherto, no complete mitogenomic data was reported in the family Meropidae, which has more than 20 species (Dickinson, 2003). Moreover, only six complete mitochondrial genome sequences (*Eurystomus orientalis*, *Halcyon coromanda*, *H. pileata*, *H. smyrnensis*, *Todiramphus sanctus* and *Upupa epops*) were available (Pacheco *et al*., 2010; Park *et al*., 2015a, b; Pratt *et al*., 2009; Qu *et al*., 2016; Zou *et al*., 2014) in the order Coraciiformes, which comprises over 250 species (Biokids, 2016).

Two subspecies (*M. v. viridis* and *M. v. americanus*) were generally recognized (Sibley and Monroe, 1990, 1993; Dickinson, 2003). However, Collar (2011) elevated the subspecies *M. v. americanus* to species Rufous-crowned Bee-eater *M. americanus* based on the study of Marks *et al*. (2007), which reported a 3.8% genetic divergence between *viridis* and *americanus* based on ND2 gene but made no suggestion for species divergence of two subspecies. Recently, *M. americanus* was treated as a distinct species by the IUCN (del Hoyo *et al*., 2014). Therefore, disputes remain as to the species placement of *M. americanus* (Sibley and Monroe, 1990, 1993; Dickinson, 2003; Coller, 2011; del Hoyo *et al*., 2014). In the study, we tentatively accepted the taxonomy of Sibley and Monroe (1990, 1993).

Herein, we first presented the complete mtDNA (*M. viridis*) of avian family Meropidae and investigated the placement of *M. americanus*.

MATERIAL AND METHODS

Ethics statement

The sample collection was strictly conducted under

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national ethical guidelines (Regulations for Administration of Affairs Concerning Experimental Animals, China, 1988) for animal husbandry and humane treatment.

Sample collection

A specimen of *M. viridis* was collected from Zhangshan township, Jizhou district, Ji'an city, Jiangxi Province, China (latitude: 27.184° N, longitude: 115.048° E) and the muscle sample and the corresponding voucher specimen (zjbj4) were vouched in the Jinggangshan University.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissue using a standard phenol-chloroform extraction protocol (Sambrook *et al*., 1989). Fourteen primers combinations (Supplementary Table S1) were designed as reference to an alignment of sequences from published mitochondrial genomes of related species (*Todiramphus sanctus* and *Upupa epops*). The overlapping PCR products ranged from 727 to 2,131 bp in length. Primers used for the generation of PCR products and the sizes of the fragments are given in Supplementary Table S1.

PCR protocol and amplification condition were set as follows the study of Huang *et al*. (2016) and made several modifications. 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 (Supplementary Table S1) in an ABI 3730xl sequencer.

Sequence analysis

All amplified sequences were assembled and edited using the software package SeqMan (DNASTAR 7.1.0) (Swindell and Plasterer, 1997). The boundaries of protein-coding genes (PCGs) were predicted by homologous sequences of other related species *E. orientalis* 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, tRNA-Cys 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). The maps of the mitochondrial genome and noncoding regions of *M. viridis* (Swindell and Plasterer, 1997) was drawn by SeqBuilder (DNASTAR 7.1.0).

Subspecies identification and genetic distance analysis

Two mtDNA fragments of ND2 (GenBank No. EU166941) and Cytb (EU167003) were isolated from the same individual, which was treated as *M. viridis* (Brown *et al*., 2008). We performed online nblast and tree-based method to delimit the specific subspecies, which was identified as *M. viridis* in this study and Brown *et al*. (2008). The nblast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was done based on the complete ND2 gene sequence. The query sequence is assigned to species with 99% of identity. Two methods of building phylogenetic trees consisting of Neighbor joining (NJ) and Bayesian inference (BI) were conducted. NJ tree was used as implemented in Mega 6.0 based on an alignment of 1041bp partial ND2 genes (Supplementary Fig. S1) with Kimura two-parameter (K2P) model (Kimura, 1980). BI tree was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and following the parameters of Tu *et al*. (2012).

To investigate the placement of *americanus*, we retrieved 4 Cytb sequences representing three closely related species of *Merops*: *M. v. viridis* (KU821702) (this study), *M. leschenaulti* (KJ456339), *M. orientalis* (KJ456340) and *M. viridis* (EU167003). Genetic distance among pairs of species was calculated using Mega 6.0 with the K2P model.

RESULTS AND DISCUSSION

Genome composition and organization

The complete mitogenome of *M. viridis* is a circular molecule of 18,295bp (Genbank No. KU821702), which contains 13 PCGs, two rRNAs, 22 tRNAs and two control regions (CR and CCR) (Supplementary Fig. S1; Supplementary Table S2).

A comparison analysis performed between the newly sequenced and Coraciiformes mitochondrial genomes indicated that the genomes were similar in many respects (Arnason *et al*., 2007). The total length of the mtDNA genomes varied from 16,562 to 18,295bp (Table I). The nucleotide composition of *M. viridis* was 28.6% A, 23.4% T, 14.4% G, and 33.6% C, and a A+T bias (52%) was found in this genome, as similar to the A+T rich pattern of other avian (Table I). To investigate the nucleotide bias, skew for a given strand was calculated (Perna and Kocher, 1995). The GC and AT skew values for the mitogenome of were -0.400 and 0.108, respectively, indicating more C and A than G and T. The result was consistent with a bias against the use of G in the vertebrates (Saccone *et al*., 1999; Rahman *et al*., 2016). The AT skew and GC skew values of mitochondrial genome sequences for Coraciiformes were consistent with the rule that the AT skew was positive while the GC skew was negative in amniote mtDNA (Quinn and Wilson, 1993).

Protein-coding genes

The 13 PCGs of *M. viridis* contain NADH dehydrogenase genes (*Nd1*, *Nd2*, *Nd3*, *Nd4l Nd4*, *Nd5*

Table I.- Base composition (%) of the sequenced Coraciiformes mitochondrial genomes.

Species	Accession number	Total length (bp)	A(%)	T(%)	G(%)	C(%)	A+T content (%)	AT skew	GC skew	References
Merops viridis	KU821702	18295	28.6	23.4	14.4	33.6	52	0.108	-0.400	This study
Halcyon coromanda	NC_028177	16542	30.2	23.5	13.7	32.5	53.7	0.145	-0.407	Park *et al*., 2015a
Halcyon pileata	NC_024198	17612	30.6	23.1	13.9	32.4	53.7	0.162	-0.400	Zou *et al*., 2014
Todiramphus sanctus	NC_011712	17549	31.3	24	13.6	31.2	55.3	0.163	-0.393	Pratt *et al*., 2009
Eurystomus orientalis	NC_011716	17210	30.1	23.3	14	32.6	53.4	0.146	-0.399	Pratt *et al*., 2009
Upupa epops	NC_028178	16562	31.5	23.9	14	30.6	55.4	0.170	-0.372	Park *et al*., 2015b

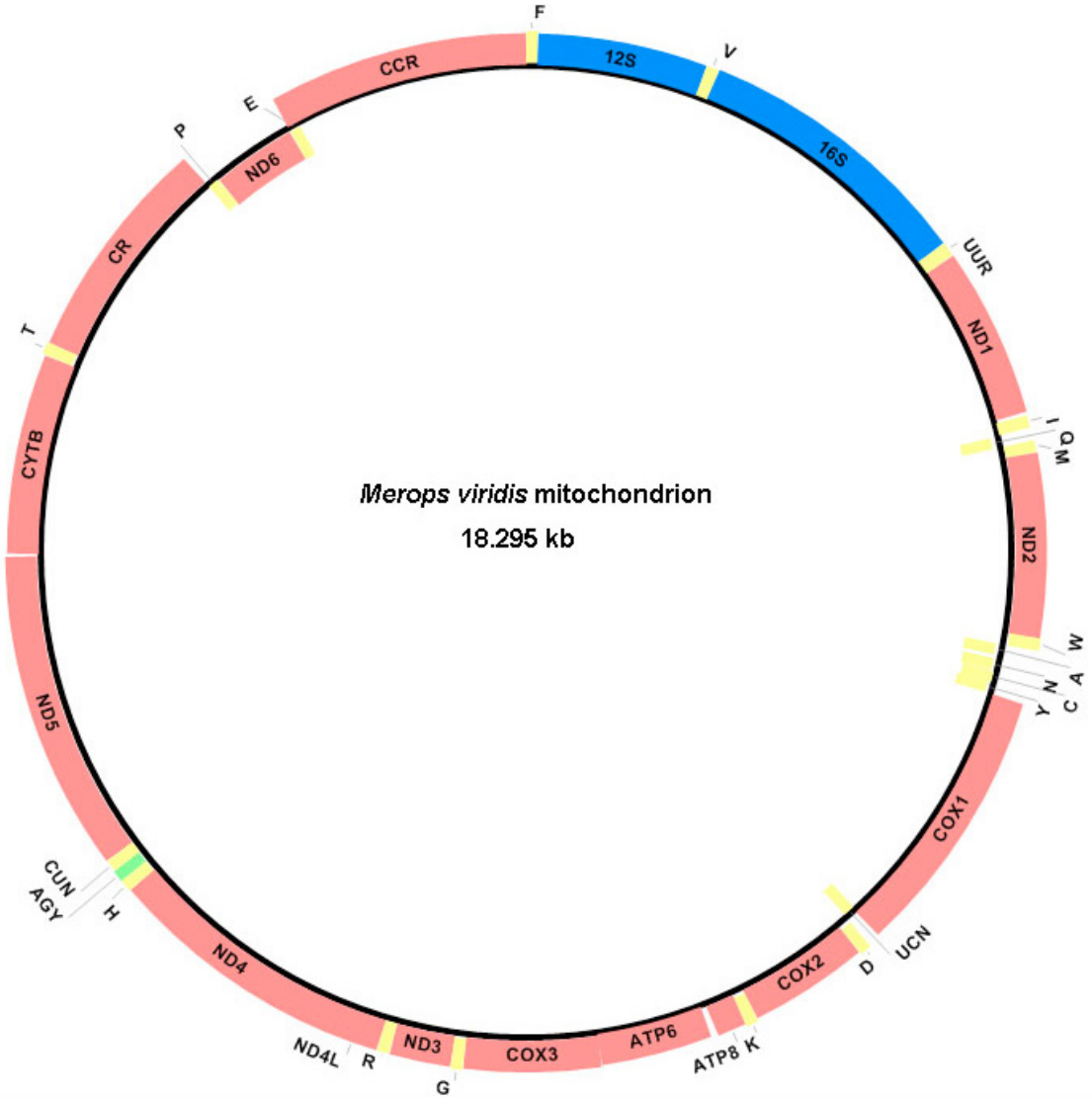

Fig. 1. Gene map of the mitochondrial genome of *Merops viridis*.

and *Nd6*), cytochrome c oxidase genes (*Cox1*, *Cox2* and *Cox3*), ATPase (*Atp8* and *Atp6*) and *Cytb* (Fig. 1). Twelve PCGs are encoded on the H-strand except *ND6*, which is located on the L-strand. All PCGs initiate with the common start codon ATG, except for *Nd3*, which begins with ATC (Supplementary Fig. S2). Five types of stop codons are used by the PCGs, including TAA for *Nd1*, *Cox2*, *Atp8*, *Atp6*, *Nd3*, *Nd4l* and *Cytb*, TAG for *Nd2 and Nd6*, AGG for *Cox1*, AGA for *Nd5*, and T for *Cox3* and *Nd4*. It is notable that an extra nucleotide is observed at position relative to ND3 sequence in many taxa of bird species (Mindell *et al*., 1998). However, the extra is undetected in the ND3 gene, as similar to some other bird species, e.g. *Geococcyx californianus*, *Lanius collurio* (Mindell *et al*., 1998).

tRNA genes and rRNA genes

As similar to most metazoans, the complete mtDNA of *M. viridis* comprises of 22 tRNAs and two rRNAs (12S rRNA and 16S rRNA) (Fig. 1). The tRNAs sequences range in size from 66 to 84 bp, and all tRNAs have the potential to fold into a complete cover leaf, with the exclusion of tRNA-Ser (AGY), which lacks the DHU stem. The 12S rRNA occurs between tRNA-Phe and tRNA-Val and 16S rRNA locates between tRNA-Val and tRNA-Leu (UUR).

Noncoding regions and gene rearrangement

The CR (1289bp) is located between tRNA-Thr and tRNA-Pro, whereas CCR (1490bp) is flanked by tRNA-Glu and tRNA-Phe (Fig. 1). The CR is comprised of three domains, corresponding to the highly variable peripheral domain I (nt 1-545) and III (nt 986-1289) and to the central conserved domain II (nt 546-985) (Fig. 2). In Domain I, extended termination associated sequences 1 (ETAS1) is observed in position nt 148-225 (Fig. 2). Four conserved sequence boxes (C, D, E and F) are identified in Domain II. CBS1 (nt 1058-1079) occurred in Domain III (Fig. 2). High sequence similarity appears between CR and CCR. A 484bp tandem repeat appears in Part A of CR and CCR (Fig. 2). Interestingly, the 7-bp short sequence motif (CAACAAA) repeats 15 times in Part B (nt 1293-1397) (Fig. 2), and the repeated motif is also found in other species (Moum and Bakke, 2001; Zou *et al*., 2014). Additionally, two noncoding regions (CR and CCR) are also found in the other voucher specimen (zjbj5) of *M. viridis*.

The mitochondrial gene order of chicken (*Gallus gallus*) was treated as typical avian gene order (Gibb *et al*., 2007; Song *et al*., 2015). Mindell *et al*. (1998) discovered a new mitochondrial gene rearrangement in *Falcon peregrinus*, which one more noncoding sequence was found between tRNA-Glu and tRNA-Phe genes except the original control region between tRNA-Thr and tRNA-Pro. In this study, we first discovered the rearrangement of CCR in order Coraciiformes (Fig. 1) and the results were similar to several representatives of Passeriformes, Procellariiformes, Cuculiformes, Piciformes and Psittaciformes (Mindell *et al*., 1998; Eberhard *et al*., 2001; Abbott *et al*., 2005). The sequence of high similarity between CR and CCR found in *M. viridis* also supporting the duplication hypothesis (Fig. 2), which was also found in the study of *Accipiter virgatus* (Song *et al*., 2015). The short repeated motif (CAACAAA) was found in mitochondrial control regions of *Alca torda* and *H. pileata* rather than that of *H. coromanda* (Supplementary Fig. S2). However, no relationships were found in these species.

Subspecies identification and genetic distance

The nblast, NJ and BI trees show that sequences EU166941 (Brown *et al*., 2008) and KU821702 (this study) should be assigned to *M. v. americanus* and *M. v. viridis*, respectively (Supplementary Fig. S1).

The intraspecies genetic distance for *M. v. viridis* and *M. v. americanus* is higher than that between *M. v. viridis* vs *M. leschenaulti* (Table II). This points towards *M. viridis* is a species *M. americanus*.

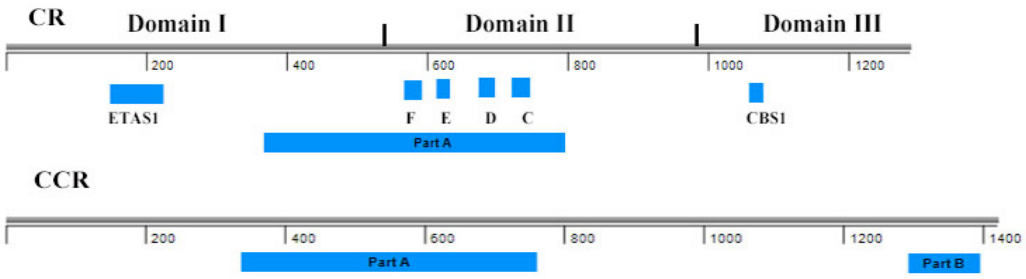

Fig. 2. Schematic representation of the organization of the *Merops viridis* control regions (CR and CCR). ETAS-extended termination-associated sequences; F through C boxes-conserved sequence boxes in the central domain; CSB-conserved sequence block; Part A-nt sequence

Table II.- Species genetic distances (%) of four related taxa based on Cytb gene.

	M. v. viridis	*M. leschenaulti*	*M. orientalis*	*M. v. americanus*
M. v. viridis (1143bp)				
M. leschenaulti (1053bp)	5.28			
M. orientalis (1143bp)	7.38	7.18		
M. v. americanus (1143bp)	6.22	5.50	7.18	

Methods of genetic divergence based on mtDNA play an important role in investigating species designation (Huang *et al*., 2016). In the present study, the genetic distance analysis showed that the intraspecific genetic divergence (*viridis* and *americanus*) was higher that the interspecific divergence. Moreover, morphological differentiations were detected between nominotypical *viridis* and *americanus*, which had the blue of the throat and upper breast reduced to a slight tinge spreading the malar area (Collar, 2011). The nominal subspecies *viridis* mainly ranges southern China and South-East Asia to the Greater Sundas and the subspecies *americanus* is confined to the Philippines (Collar, 2011; Zheng, 2011). Combined the morphological, distribution and genetic divergence data, we confirm that the *M. americanus* should be treated as full species status, supporting the taxonomic arrangement of *americanus* by the IUCN (del Hoyo *et al*., 2014).

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of the manuscript.

Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2017.49.1.79.84

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