Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA, Early Online: 1–2
 DOI: 10.3109/19401736.2014.926503

informa
healthcare

MITOGENOME ANNOUNCEMENT

Extreme sequence divergence between mitochondrial genomes of two subspecies of White-breasted Wood-wren (*Henicorhina leucosticta*, Cabanis, 1847) from western and central Panamá

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Abstract

Prior studies of mitochondrial variation in White-breasted Wood-Wrens (*Henicorhina leucosticta*) have suggested that populations in South American and Mesoamerica might represent multiple species. Here we report the complete mitochondrial genomes from two individuals of *H. leucosticta*, representing the Panamanian subspecies *pittieri* and *alexandri*. The two sequences were 16,721 and 16,726 base pairs in size with both genomes comprised of the usual 22 tRNA genes, 2 rRNA genes, 13 protein-coding genes, and one displacement loop region in the standard avian order. Uncorrected pairwise divergence between mitogenome features was high, with the highest divergence occurring in protein-coding genes (average = 8.2%), followed by control region (6.7%). RNA features had lower pairwise divergences (average tRNA = 4.3%, average rRNA = 2.3%). The protein-coding ATPase 6 gene had a different stop codon between these two specimens. The high level of sequence variation between these subspecies suggests that Mesoamerican *H. leucosticta* might be comprised of multiple species. We urge a full phylogeographic survey of this widespread Neotropical forest bird.

Keywords

Mitogenome, multiple species, Troglodytidae

History

Received 1 May 2014
Accepted 9 May 2014
Published online 18 June 2014

The White-breasted Wood-wren (*Henicorhina leucosticta*; Cabanis, 1847) is an understory insectivorous bird found throughout lowland rainforests from southern Mexico to Guyana and northern Peru. Thirteen subspecies of *H. leucosticta* have been described (Dickerman, 1973; Paynter & Vaurie, 1960), and a recent analysis of mitochondrial ATP-synthase 6 and 8 sequence variation suggests that three species might be involved – one from Amazonia, one from western Ecuador, and one from Mesoamerica (Dingle et al., 2006). Although nine of the 13 recognized subspecies are found in Middle America, Dingle et al. (2006) found little divergence between samples from Belize and Panamá, suggesting pervasive gene flow across Mesoamerica. However, that study did not include the subspecies *pitteri* endemic to Pacific Costa Rica and western Panamá. As part of our survey of genetic variation across avian suture zones in Panamá, we sequenced the mitogenomes of two individuals of *H. leucosticta* representing two subspecies: *pittieri*, (Chiriquí Province) and *alexandri* (Panamá Province).

Genomic DNA was isolated from tissues archived in the Smithsonian Tropical Research Institute Cryological Collections: MJM4821 (*pittieri*) and MJM1044 (*alexandri*). Mitochondrial genomes were obtained as by-product of next generation sequencing of ultra-conserved elements (UCEs; Faircloth et al., 2012; Miller et al., 2014). Contigs were generated in Velvet 1.2 (k-mer: 95; Zerbino & Birney, 2008) using the phyluce bioinformatics package (https://github.com/faircloth-lab/phyluce) from paired-end 150 base pair MiSeq (Illumina, San Diego, CA). The largest contig (over 16,000 base pairs) was identified as mitochondrial DNA, and an initial annotation was completed using DOGMA (Wyman et al., 2004) and subsequently inspected by eye. We then mapped all reads onto the draft genomes using Bowtie 2.2.1 (Langmead & Salzberg, 2012) to recover the consensus sequence of the entire mitogenome. A total of 36,673 (MJM 4821) and 21,718 (MJM 1044) reads were mapped to both individuals with an average coverage of 185 and 287 reads respectively. Genbank accessions for these sequences are KJ719074 and KJ746107. The length size of the sequences was 16,721 bp for MJM4821 and 16,726 for MJM1044, and both had the standard avian mitogenome order (Table 1).

We calculated uncorrected sequence variation based on Muscle alignments implemented in Geneious 7.0.6. (Biomatters, http://www.geneious.com). Average sequence divergence for coding regions ranged from 6.2% ~ 11.3% (average = 8.2%). Furthermore, the ATP6 gene shows a change in stop codon between the two sequences. Variation among non-coding regions was lower. Variation among the D-loop was 6.7%, while average tRNAs features varied by 4.3% and the average ribosomal RNA sequence variation was 2.3%. This high level of protein-coding sequence variation is above the levels typically reported within species for birds (Kerr et al., 2007; Price, 2008), and suggests the presence of multiple species of *H. leucosticta* within Mesoamerica. We encourage future surveys of mitochondrial DNA sequence variation across the entire range of *H. leucosticta*, and we hope that these mitogenomes will inform future studies of species limits and evolutionary relationships within the White-breasted Wood-wren complex.

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Table 1. Characteristics of the mitochondrial genome of two *H. leucosticta* subspecies from Panamá. Parenthetical values refer to specimen MJM1044 (Panamá Province); otherwise features were identical.

Code	Amino Acid	Start	Stop	Size	Spacer (+) or Overlap (−)	Direction	Start Codon	Stop Codon
F	tRNA-Phe	1	69	69	0	F		
	12S rRNA	70	1045	976	0	F		
V	tRNA- Val	1046	1115	70	0	F		
	16S rRNA	1116	2707	1592 (1593)	4	F		
L	tRNA-Leu	2712 (2713)	2786 (2787)	75	14	F		
	ND1	2801 (2802)	3778 (3779)	978	10 (11)	F	ATG	AGA
I	tRNA-Ile	3789 (3791)	3863 (3864)	75 (74)	6	F		
Q	tRNA-Gln	3870 (3871)	3940 (3941)	71	−1	R		
M	tRNA-Met	3940 (3941)	4008 (4009)	69	0	F		
	ND2	4009 (4010)	5049 (5050)	1041	0 (−1)	F	ATG	TAA
W	tRNA-Trp	5050	5119	70	1	F		
A	tRNA-Ala	5121	5189	69	6	R		
N	tRNA-Asn	5196	5268	73	0	R		
C	tRNA-Cys	5269	5335	67	−1	R		
Y	tRNA-Tyr	5335	5405	71	1	R		
	COX I	5407	6957	1551	−9	F	ATG	AGG
S	tRNA-Ser (UCN)	6949	7021	73	5	R		
D	tRNA-Asp	7027	7095	69	10	F		
	COX II	7106	7789	684	1	F	ATG	TAA
L	tRNA-Lys	7791	7861 (7859)	71 (69)	1	F		
	ATP 8	7863 (7861)	8030 (8028)	168	−10	F	ATG	TAA
	ATP 6	8021 (8019)	8704 (8702)	684	6	F	ATG	TAA (TAG)
	COX III	8711 (8709)	9494 (9492)	784	0	F	ATG	T−−
G	tRNA-Gly	9495 (9493)	9563 (9561)	69	0	F		
	ND3	9564 (9562)	9914 (9912)	351	1	F	ATG	TAA
R	tRNA-Arg	9916 (9914)	9984 (9982)	69	1	F		
	ND4L	9986 (9984)	10,282 (10,280)	297	−7	F	ATG	TAA
	ND4	10,276 (10,274)	11,653 (11,651)	1378	0	F	ATG	T−−
H	tRNA-His	11,654 (11,652)	11,723 (11,721)	70	1	F		
S	tRNA-Ser	11,725 (11,723)	11,788 (11,786)	64	0	F		
L	tRNA-Leu 2	11,789 (11787)	11,859 (11,857)	71	0	F		
	ND5	11,860 (11,858)	13677 (13,675)	1818	11 (12)	F	ATG	AGA
	Cytb	13,689 (13,688)	14,831 (14,830)	1143	3	F	ATG	TAA
T	tRNA-Thr	14,835 (14,834)	14,903 (14,902)	69	9 (8)	F		
P	tRNA-Pro	14,913 (14,911)	14,981 (14,979)	69	14 (18)	R		
	ND6	14,996 (14,998)	15,514 (15,516)	519	0	R	ATG	TAG
E	tRNA-Glu	15,515 (15,517)	15,585 (15,587)	71	0	R		
	Control Region	15,586 (15,588)	16,721 (16,726)	1136 (1139)	0	F		
	Total genome size	16,721 (16,726)						

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article. This work was funded by the Smithsonian Institution and the Smithsonian Tropical Research Institute Office of Fellowships.

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