Mitochondrial DNA

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MITOGENOME ANNOUNCEMENTS

Complete mitochondrial genome recovered from the gut metagenome of overwintering monarch butterflies, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae, Danainae)

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Abstract

We present a 15,314 bp mitochondrial genome (mitogenome) sequence from monarch butterflies overwintering in Mexico. The complete mitogenome was generated by next generation sequencing techniques and was reconstructed by iterative assembly of reads from a metagenomic study of pooled butterfly gut DNA. The mitogenome codes for 13 putative protein coding genes, 22 tRNA genes, the large and small rRNA genes, and contains the A + T-rich sequence corresponding to the control region. The consensus sequence presented here has a depth of coverage of 142-fold and only three putative single nucleotide polymorphisms could be detected. The recovered *D. plexippus* mitogenome represents the second analyzed for the subfamily Danainae and accordingly, the closest available sequenced mitogenome was found to be the one corresponding to *Euploea mulciber* (Lepidoptera: Nymphalidae, Danainae).

Keywords

Danaus plexippus, lepidoptera, metagenomics, mitochondrial genome, Nymphalidae

History

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We present a mitogenome sequence of the monarch butterfly with the GenBank accession number KC836923. The mitogenome was designated with the acronym DPMMX (***D****anaus* ***p****lexippus* **m**itogenome -**Mex**ico) as it is a consensus sequence obtained by metagenomic sequencing of gut DNA from overwintering butterflies.

Butterflies were collected during January 2010 from the Sierra Chivati-Huacal at the Monarch Butterfly Biosphere Reserve in Mexico. Total DNA was purified from the guts of three female butterflies using the UltraClean microbial DNA kit (MoBio Laboratories, Inc., Carlsbad, CA). Metagenomic sequencing was performed on an Illumina GAIIx platform producing 2.51 Gbp. Reads were assembled using Velvet 1.2.07 (Zerbino & Birney, 2008.). A set of 13 contigs were predicted by BLAST searches to be of mitochondrial origin. Gaps were closed iteratively by mapping and reassembling reads to these contigs using Maq 0.7.1 (Li et al., 2008) and Velvet. Additionally, PCRs were performed to amplify and sequence regions within gaps or homopolimeric regions and for validation. The primers used were MDs10-modified 5′-AACAGGATCAAA TAATCCATTAGG-3′ and MDs11 5′-AAATTACCTTAGGGAT AACAGCG-3′, MDs14 5′-TCGTGGATTATCAATTATTAAAC AGATTCC-3′ and MDs2L-modified 5′-GCTGTAATACCTACT GCTC-3′ (Cameron & Whiting, 2008) and 12SB 5′-AAACTAGG ATTAGATACCC-3′ (Skerratt et al., 2002) and 16SB-modified 5′-CACCGGTTTGAACTCAGATCA-3′ (Bybee et al., 2004). Open reading frames were predicted using GeneMark.hmm2.0 (Besemer & Borodovsky, 1999). Features were manually verified using Artemis (Carver et al., 2005).

The length of DPMMX was determined to be 15,314 bp and its nucleotide composition is biased exhibiting 81.4% A + T content. The average coverage of the circular genome was 142-fold. Only three candidate single nucleotide polymorphisms (SNPs) were detected by Maq (T323, C1712 and G1732). The mitogenome codes for 13 protein coding genes, 22 tRNA genes, the large and small rRNA genes, and contains a control region with an A + T content of 94.5% exhibiting some conserved lepidopteran motifs including the ‘‘ATAGA’’ and 19 bp poly (T) stretch. The protein encoding genes have typical mitochondrial ATN start codons, except for cox1, which contains the unusual CGA codon and which also shows an incomplete stop codon.

Blast searches were performed using DPMMX as a query against the MonarchBase (Zhan & Reppert, 2013), a database repository for the data of the *D. plexippus* genome (Zhan et al., 2011). A contig of 24,802 bp could be detected with accession number AGBW01003356.1 corresponding to the non-described mitogenome. This unedited contig shows redundancy at its ends and should be circular and its sequence is conserved with DPMMX albeit with dissimilarities. Major differences are an additional 1954 bp region containing repetitive TA bases and a non-conserved sequence not present in DPMMX and the absence of a 221 bp region conserved among other sequenced lepidopteran mitogenomes including DPMMX. Differences could be attributed to natural variation, sequencing errors or misassembles at homopolimeric regions. In conclusion, the mitogenome of *D. plexippus* presents a conserved structure and shows few polymorphisms in accordance with the low genetic differentiation reported for monarch butterflies (Brower & Boyce, 1991; Brower & Jeansonne, 2004; Lyons et al., 2012).

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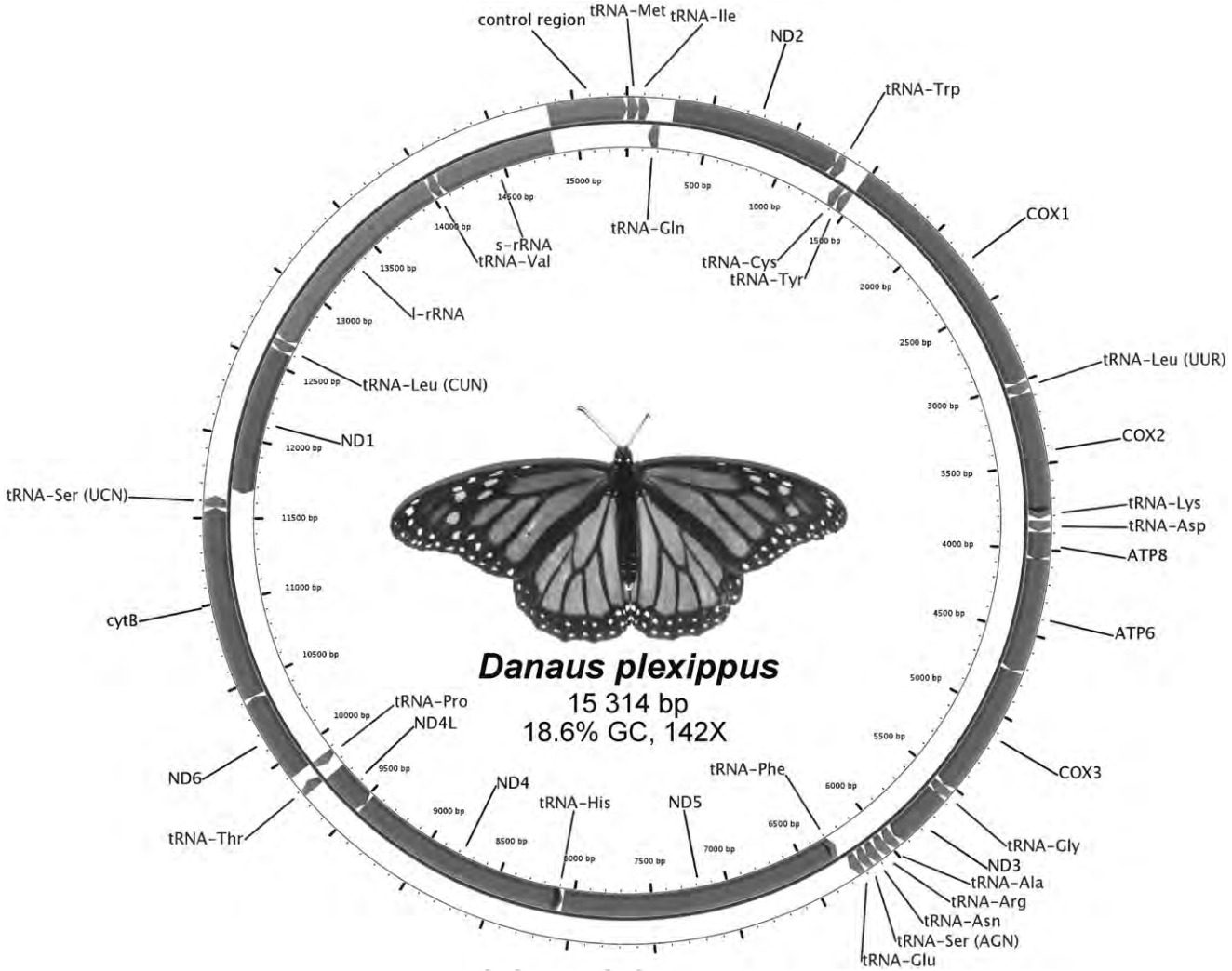

Figure 1. Circular map of the DPMMX version of the mitochondrial genome of *Danaus plexippus*.

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Declaration of interest

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