

RESEARCH ARTICLE

 OPEN ACCESS

The complete mitochondrial genome of the Bermuda buckeye butterfly *Junonia coenia bergi* (Insecta: Lepidoptera: Nymphalidae)

Melissa J. Peters and Jeffrey M. Marcus

Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

ABSTRACT

The Bermuda buckeye, *Junonia coenia bergi*, is the only butterfly endemic to Bermuda, but is largely unstudied. Whole-genome Illumina sequencing was used to obtain a complete circular mitochondrial genome sequence of 15,221 bp consisting of 22 tRNAs, 13 protein-coding genes, 2 rRNAs and a control region. Mitogenome structure and organization was found to be very similar to that of other *Junonia* butterfly mitogenomes. Excluding ambiguous nucleotides, the *J. coenia bergi* mitogenome is 99.1% identical to the *J. coenia coenia* mitogenome. Parsimony and maximum-likelihood phylogenetic reconstruction revealed the monophyly of subfamily Nymphalinae, genus *Junonia*, and species *J. coenia*.

ARTICLE HISTORY

Received 17 February 2016

Revised 21 February 2016

Accepted 26 February 2016

KEYWORDS

Bermuda buckeye butterfly;
illumina sequencing;
Junonia coenia bergi;
mitochondrial genome;
Nymphalinae

The common buckeye, *Junonia coenia*, is a model system for insect physiology (Kremen & Nijhout 1998), development (Keys et al. 1999) and larval host-plant specialization (Knerl & Bowers 2013). *Junonia coenia* includes 3 North American subspecies. The grey or northern buckeye, *Junonia coenia grisea*, lives in California, Mexico, Oregon, Nevada and Arizona (Austin & Emmel 1998) and exhibits a 0.92% barcode divergence from *J. coenia coenia*, which lives in eastern North America, Cuba and the Bahamas (Gemmell & Marcus 2015). *Junonia coenia bergi*, which is endemic to Bermuda (Avinoff 1926), is largely unstudied. Here, we report the complete mitochondrial genome sequence of *J. coenia bergi* (GenBank KU524879).

Junonia coenia bergi specimen BERCLL2 was netted at Lover's Lake, St. George's, Bermuda (coordinates: N 32.3678, W 64.7095) on 25 September 2015 by S. R. Smith. The specimen was pinned, spread and has been deposited in the collection of the Bermuda Aquarium, Museum and Zoo (BAMZ voucher 2015-293-017). DNA was prepared (McCullagh & Marcus 2015) and sequenced by Illumina MiSeq (San Diego, California). Overall, 2,634,992 paired reads (total 1.2 Gb) were assembled in Geneious 8.1.8 to a *J. lemonias* reference mitogenome (KP941756) (McCullagh & Marcus 2015). Annotation was performed with reference to *J. lemonias* and *J. coenia coenia* (South Carolina, KT380025) mitogenomes (Teng et al. 2015). tRNAs were evaluated using RNA Structure software (Reuter & Mathews 2010).

The circular mitochondrial genome of *J. coenia bergi* consists of 15,221 bp compared to 15,222 bp for *J. coenia coenia* (Teng et al. 2015), with similar nucleotide compositions (*bergi/coenia*: 39.5%/39.6% A, 11.7%/11.6% C, 7.9%/7.7% G, 40.9%/41.1% T). Excluding ambiguous nucleotides, these mitogenomes are 99.1% identical (with only 0.15% barcode divergence). Gene composition and arrangement are identical to other butterfly mitogenomes with 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and a control region (McCullagh & Marcus 2015).

J. coenia bergi COI has an aberrant start codon (CGA) which is typical of insects. Three genes (COI, COII, NAD4) have aberrant single-nucleotide (T) stop codons, as seen in other *Junonia* mitogenomes (McCullagh & Marcus 2015). *Junonia coenia bergi* lacks aberrant stop codons in CYTB and NAD2 that are present in *J. coenia coenia*. All but one tRNA have standard cloverleaf secondary structures. In trnS (AGN), the dihydrouridine arm is replaced by a loop as seen in many arthropods (McCullagh & Marcus 2015). The rRNAs (774 bp 12S and 1330 bp 16S) are composed of 84% AT, while the putative control region (332 bp) is 93.1% AT.

We reconstructed a phylogeny using mitogenomes from *J. coenia bergi*, six other *Junonia* and 21 other Nymphalid butterfly species. Two mitogenomes from *J. iphita* have been described (Vanlalruati et al. 2015a, 2015b), but with unpublished assemblies, so we re-assembled the mitogenomes (KU577289, KU577290) from sequence read archives

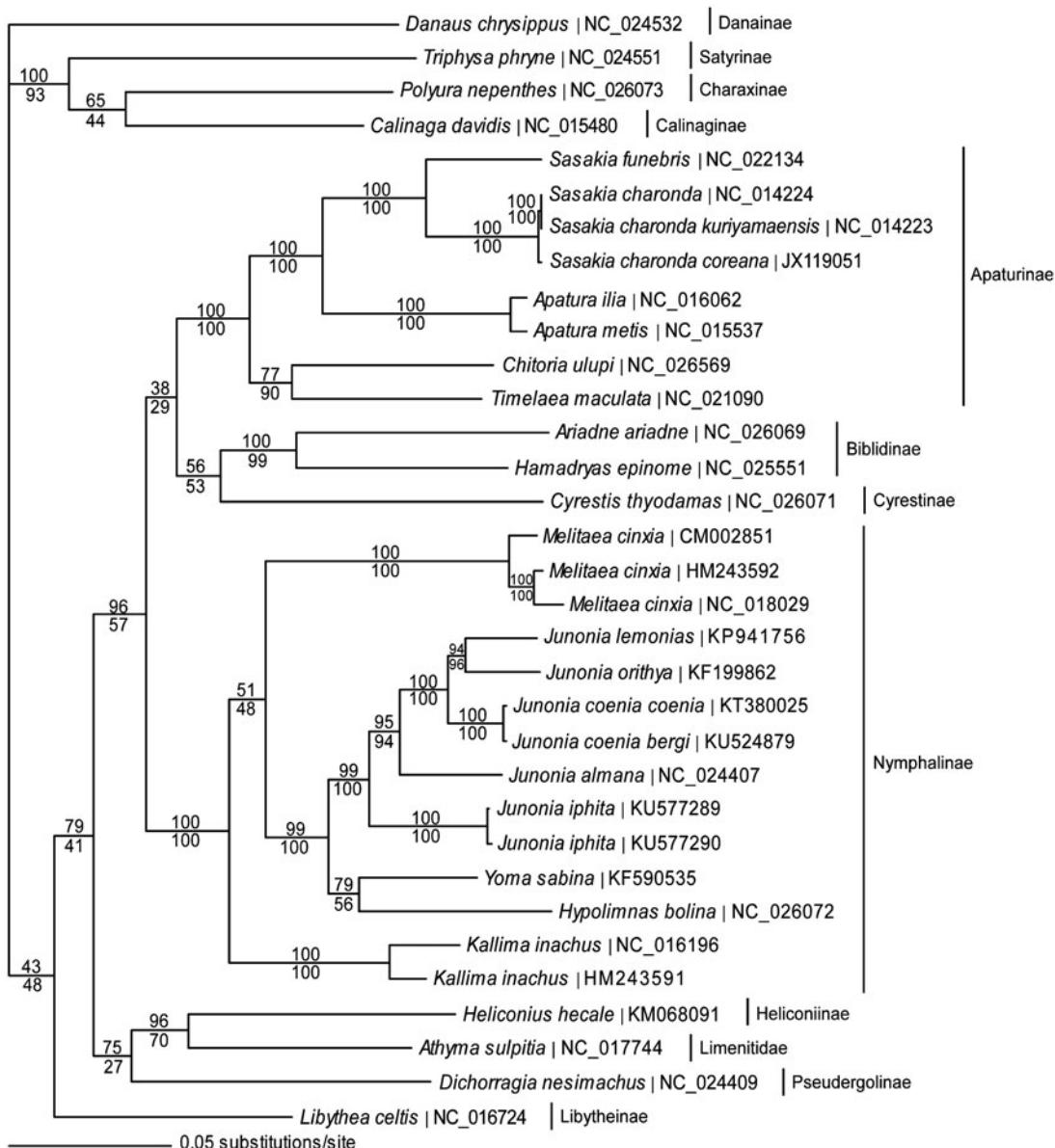


Figure 1. Maximum-likelihood phylogeny (GTR + I + G model, likelihood score 136451.14) of *Junonia* and other Nymphalid butterflies based on one million random addition heuristic search replicates (with tree bisection and reconnection) of aligned complete mitochondrial genomes. One million maximum parsimony heuristic search replicates produced an identical tree topology (parsimony score 26,710 steps). Numbers above each node are maximum-likelihood bootstrap values and numbers below each node are maximum parsimony bootstrap values (each from one million random fast addition search replicates).

(SRP053322, SRP053690). Mitogenome sequences were aligned in CLUSTAL Omega (Sievers et al. 2011) and analysed by parsimony and maximum likelihood in PAUP* 4.0b8/4.0d78 (Swofford 2002) (Figure 1). The subspecies of *J. coenia* are closely associated within the monophyletic genus *Junonia*.

Acknowledgements

We thank Robbie Smith (Bermuda Natural History Museum (BAMZ)) and Debbie Tsuyuki (Next Generation Sequencing Platform, Manitoba Institute of Child Health). Bonnie McCullagh, Ashley Haverstick, and Melanie Lalonde provided helpful comments on the manuscript. This is Contribution #239, Bermuda Biodiversity Project (BBP), Bermuda Aquarium, Natural History Museum and Zoo, Department of Conservation Services.

Disclosure statement

The authors report no conflicts of interest, and are solely responsible for this paper.

Funding information

This work received support from the University of Manitoba Centre for the Advancement of Teaching and Learning and NSERC under Grant RGPIN386337-2011.

References

- Austin GT, Emmel JF. 1998. New subspecies of butterflies (Lepidoptera) from Nevada and California. In: Emmel TC, editor. Systematics of Western North American butterflies. Gainesville, FL: Marioposa Press. p. 501–522.
- Avinoff A. 1926. Descriptions of some new species and varieties of Rhopalocera in the Carnegie Museum. Ann Carnegie Mus. 16: 355–374.
- Gemmell AP, Marcus JM. 2015. A tale of two haplotype groups: Evaluating the new world *Junonia* ring species hypothesis using the distribution of divergent COI haplotypes. Syst Entomol. 40: 532–546.

- Keys DN, Lewis DL, Selegue JE, Pearson BJ, Goodrich LV, Johnson RJ, Gates J, Scott MP, Carroll SB. 1999. Recruitment of a *hedgehog* regulatory circuit in butterfly eyespot evolution. *Science*. 283:532–534.
- Knerl A, Bowers MD. 2013. Incorporation of an introduced weed into the diet of a native butterfly: consequences for preference, performance and chemical defense. *J Chem Ecol*. 39:1313–1321.
- Kremen, C, Nijhout, HF. 1998. Control of pupal commitment in the imaginal disks of *Precis coenia* (Lepidoptera: Nymphalidae). *J Insect Physiol*. 44:287–296.
- McCullagh BS, Marcus JM. 2015. The complete mitochondrial genome of Lemon Pansy, *Junonia lemonias* (Lepidoptera: Nymphalidae: Nymphalinae). *J Asia-Pacific Ent*. 18:749–755.
- Reuter JS, Mathews DH. 2010. RNAstructure: software for RNA secondary structure prediction and analysis. *BMC Bioinformatics*. 11:129.
- Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, et al. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Syst Biol*. 7:539. doi:10.1038/msb.2011.75.
- Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (*and Other Methods). Version 4. Sunderland (MA): Sinauer Associates.
- Teng XX, Xie ZH, Zhang Y. 2015. The complete mitochondrial genome of the common buckeye *Junonia coenia* (Insecta: Lepidoptera: Nymphalidae). Mitochondrial DNA. 1–2. [Epub ahead of print]. doi:10.3109/19401736.2015.1089496.
- Vanlalruati C, De Mandal S, Guruswami G, Nachimuthu SK. 2015a. Complete mitochondrial genome of Chocolate Pansy, *Junonia iphita* (Lepidoptera: Nymphalidae: Nymphalinae). Mitochondrial DNA. 1–2. [Epub ahead of print]. doi:10.3109/19401736.2015.1033701.
- Vanlalruati C, De Mandal S, Guruswami G, Nachimuthu SK. 2015b. Illumina based whole mitochondrial genome of *Junonia iphita* reveals minor intraspecific variation. *Genomics Data*. 6:280–282.