The complete mitochondrial genome and phylogenetic placement of *Apis nigrocincta* Smith (Insecta: Hymenoptera: Apidae), an Asian, cavity-nesting honey bee

Amin Eimanifar\(^a\), Rebecca T. Kimball\(^b\), Edward L. Braun\(^b\), Stefan Fuchs\(^c\), Bernd Grünewald\(^d\) and James D. Ellis\(^e\)

\(^a\)Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA; \(^b\)Department of Biology, University of Florida, Gainesville, FL, USA; \(^c\)Institut für Bienenkunde, Polytechnische Gesellschaft, Goethe-Universität Frankfurt am Main, Oberursel, Germany

**ABSTRACT**

The complete mitochondrial genome of *Apis nigrocincta* was sequenced. The mitochondrial genome is a circular molecule of 15,855 bp, including 37 classical eukaryotic mitochondrial regions and an A+T-rich region. Gene directions and arrangements are similar to those of other *Apis* mitogenomes. Most genes initiated with ATT; though ATG and ATA were also used as start codons. Twelve of 13 protein-coding genes terminated with TAA, though ND2 terminated with TAG. Four PCG genes, eight tRNAs and both rRNAs were encoded on the heavy strand while all others were encoded on the light strand (9 PCGs and 14 tRNAs). Overall, the GC content composed 15.6% of the mitogenome. All of the 22 tRNA genes, ranging from 66 to 114 bp, have a typical cloverleaf structure. A phylogenetic tree showed that *A. nigrocincta* clustered closest to *A. cerana*. The complete mitogenome of *A. nigrocincta* provides essential information on the biogeography and evolution of this Asian honey bee species.
Twenty-two tRNA genes were identified between the rRNA and PCGs, ranging in size from 59 to 77 bp. All tRNAs folded into a typical cloverleaf-shaped secondary structure as identified by tRNAscan-SE (Lowe & Eddy 1997). The sizes of the small ribosomal RNA (12S rRNA) and large ribosomal RNA (16S rRNA) genes were 782 and 1331 bp, respectively.

The phylogenetic position of *A. nigrocincta* with inclusion of 6 other *Apis* species was estimated using RaxML 8.0.20 (Stamatakis 2006) with 1000 bootstrap replicates using 13 PCGs and two rRNAs. *Apis nigrocincta* clustered with *A. cerana* with high bootstrap support (Figure 1). The phylogenetic analysis was consistent with morphological and molecular evidence, indicating that *A. nigrocincta* has similarities with *A. cerana* (Hadisoesilo & Otis 1996; Raffiudin & Crozier 2007).

The maximum p-distance was between *A. nigrocincta* and *A. andreniformis* (0.16) and the minimum between *A. nigrocincta* and *A. cerana* (0.07). In conclusion, the complete mitogenome of *A. nigrocincta* provides essential and important molecular data for understanding the evolution and biogeography of *Apis*.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**Funding**

This project was financed through a cooperative agreement provided by the United States Department of Agriculture and Animal and Plant Health Inspection Service (USDA-APHIS) under cooperative agreement 16-8130-0414-CA.

**References**

Arias MC, Sheppard WS. 2005. Phylogenetic relationships of honey bees (Hymenoptera: Apinae: Apini) inferred from nuclear and mitochondrial DNA sequence data. Mol Phylogenet Evol. 37:22–35.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 19:455–477.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina Sequence Data. Bioinformatics. 30:2114–2120.

Damus MS, Otis GW. 1997. A morphometric analysis of *Apis cerana* F. and *Apis nigrocincta* Smith populations from southeast Asia. Apidologie. 28:309–323.

Deatherage DE, Barrick JE. 2014. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using bresq. Methods Mol Biol. 1151:165–188.

Eimanifar A, Kimball RT, Braun EL, Ellis JD. 2016. The complete mitochondrial genome of the Cape honey bee, *Apis mellifera capensis* Esch. (Insecta: hymenoptera: apiidae). Mitochondrial DNA Part B. 1:817–819.

Hadiososilo S, Otis GW. 1996. Drone flight times confirm the species status of *Apis nigrocincta* Smith, 1861 to be a species distinct from *Apis cerana* F, 1793, in Sulawesi, Indonesia. Apidologie. 27:361–369.

Hepburn HR, Radloff SE, editors. 2011. Honeybees of Asia. Heidelberg, Dordrecht, London, New York: Springer.

Langmead B, Salzberg S. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods. 9:357–359.

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25:955–964.

Raffiudin R, Crozier RH. 2007. Phylogenetic analysis of honey bee behavioral evolution. Mol Phylogenet Evol. 43:543–552.

Stamatakis A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 22:2688–2690.