

The complete mitochondrial genome of the yellow coloured honeybee *Apis mellifera* (Insecta: Hymenoptera: Apidae) of New Zealand

Ikumi Nakagawa^a, Mito Maeda^a, Mao Chikano^a, Hisashi Okuyama^a, Robert Murray^b and Jun-Ichi Takahashi^a

^aFaculty of Life sciences, Kyoto Sangyo University, Kyoto, Japan; ^bTai Tokerau Honey Ltd, Kaitaia, New Zealand

ABSTRACT

The complete mitochondrial genome of the yellow coloured honeybee *Apis mellifera* from North Island, New Zealand was analyzed using next-generation sequencing. The mitochondrial genome was a 16,349bp circular molecule and was predicted to contain 13 protein-coding genes (PCGs), 22 tRNA genes and two rRNA genes. The initiation codon ATA was found in two genes, ATG in four genes, ATT in six genes, and ATC in one gene, while the termination codon TAA was observed in all the PCGs. Phylogenetic analysis using the sequence of 23 closely related taxa suggested a sister relationship with the Italian strain *A. mellifera ligustica*.

ARTICLE HISTORY

Received 8 December 2017
Accepted 26 December 2017

KEYWORDS



Western honeybee; Illumina sequencing; *Apis mellifera ligustica*; New Zealand; subspecies

The western honeybee, *Apis mellifera* is naturally distributed in African, the Middle East, and Europe, and is exported worldwide. The black honeybee strain *A. m. mellifera* was imported from the UK to New Zealand in 1839 (Beard 2015). Later, New Zealand further imported two subspecies, *A. m. carnica* and *A. m. ligustica*. Honeybees have been imported from abroad until the 1980s, but imports have now been prohibited to control diseases (Beard 2015). The imported honeybees were bred for beekeeping in New Zealand, but some honeybees were naturalized in the bush biome. A record from 1860 reports that New Zealand-made honey was sold by the Maori tribe. Commercial beekeeping in New Zealand began after the Langstroth's Hive was introduced in 1870 (Beard 2015).

Here, we report the complete mitochondrial genome of the yellow coloured honeybee *A. mellifera* found in New Zealand, which will be useful for phylogenetic studies involving the New Zealand honeybees and other *A. mellifera* subspecies. Adult workers of the yellow coloured *A. mellifera* were collected in March 2017 from Kaitaia, North Island, New Zealand. Genomic DNA isolated from one worker was sequenced using Illumina's HiSeq platform. The resultant reads were assembled and annotated using the MITOS web server (Germany; Bernt et al. 2013) and Geneious R9 (Biomatters, Auckland, New Zealand). A phylogenetic tree was constructed using MEGA6 (Tamura et al. 2013) and TREEFINDER (Jobb et al. 2004) using the nucleotide sequences of 13 protein-coding genes (PCGs).

The yellow coloured *A. mellifera* mitochondrial genome was found to form a closed loop that is 16,349 bp

long (AP018435). The yellow coloured *A. mellifera* mitochondrial genome represented a typical hymenopteran pattern and was similar to the common *A. mellifera* mitochondrial genome organization, comprising 13 PCGs, 22 putative tRNA genes, two rRNA genes and an A+T-rich control region. The average AT content of the *A. mellifera* mitochondrial genome was 84.91%. Similar to the honeybee mitochondrial genomes, the heavy strand encoded nine protein-coding genes and 14 tRNA genes, and the light strand encoded four protein-coding genes, eight tRNAs, and two rRNA genes. The *ATP6* and *ATP8* genes shared 19 nucleotides. Six protein-coding genes of the *A. mellifera* mitochondrial genome started with *ATT*, the *ATP6*, *COIII*, *ND4*, and *Cytb* genes started with *ATG*, the *COI* and *ND3* genes started with *ATA*, and the *ND2* gene started with *ATC*, all of which have been commonly found in the *A. mellifera* subspecies mitochondrial genome (Crozier and Crozier 1993; Gibson and Hunt 2015; Hu et al. 2015; Haddad 2015; Eimanifar et al. 2016a, 2016b; Eimanifar et al. 2017a, 2017b, 2017c; Haddad et al. 2017). The stop codon of each of these protein-coding genes was either *TAA*, similar to that in the other honeybee subspecies. All the tRNA genes typically possessed cloverleaf secondary structures, except for *Gln*, *Ser1*, and *Thr*, which lacked the arm. Phylogenetic analysis was conducted using the sequence information of 13 mitochondrial PCGs from 23 closely related taxa (Figure 1). The New Zealand yellow coloured *A. mellifera* was most closely related to the Italian subspecies *A. m. ligustica* among all the *A. mellifera* subspecies with sequence homologies ranging from 0.009 to 0.0171.

CONTACT Jun-Ichi Takahashi  jit@cc.kyoto-su.ac.jp  Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan

© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

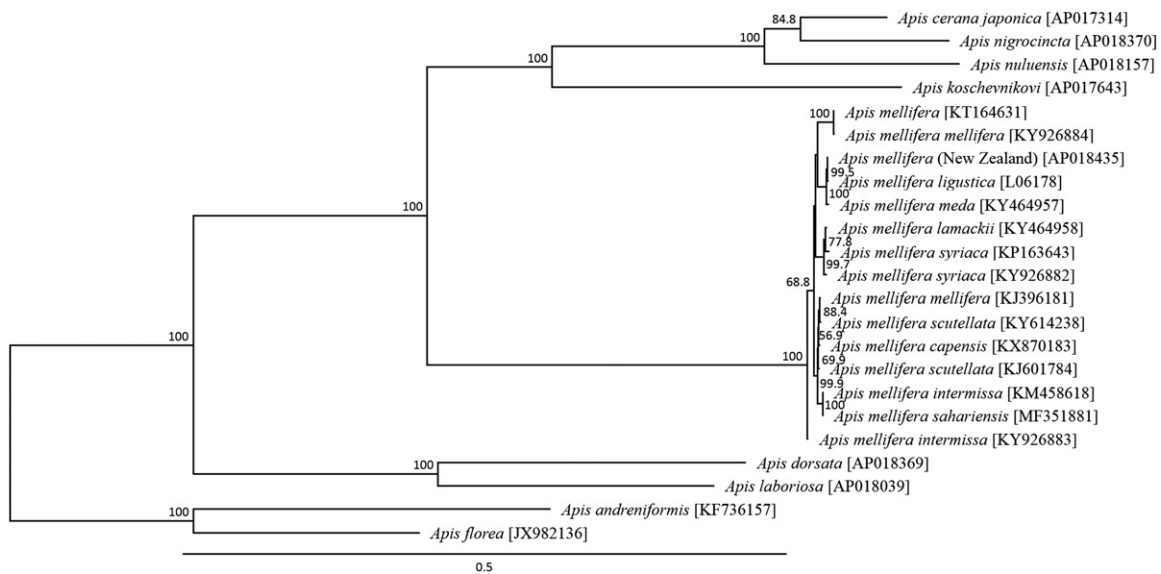


Figure 1. Phylogenetic relationships (maximum likelihood) among the species of the genus *Apis* (Hymenoptera), as determined using the mitochondrial DNA nucleotide sequences of the 13 protein-coding genes. The numbers beside the nodes are the percentages of 1,000 bootstrap values. *Apis florea*, *A. andreniformis*, *A. dorsata*, *A. laboriosa*, *A. cerana*, *A. nigrocincta*, and *A. koschevnikovi* (Takahashi et al. 2016; Takahashi et al. 2017a, 2017b, 2017c, 2017d; Wakamiya et al. 2017) were used as an outgroup. The alphanumeric terms in the parentheses indicate the GenBank accession numbers.

Disclosure statement

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

Acknowledgements

The authors are grateful to Dr. T. Kiyoshi, Mr. Kano and Mr. N. Raymond for valuable comments on the manuscript. The DNA specimen was stored in the National Museum of Nature and Science, New Zealand, accession number: NSMT-I-HYM 75315.

References

- Beard C. 2015. Honeybees (*Apis mellifera*) on public conservation lands: a risk analysis. Wellington: Department of Conservation; p. 21.
- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol Phyl Evol.* 69:313–319.
- Crozier RH, Crozier YC. 1993. The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics.* 133:97–117.
- Eimanifar A, Kimball RT, Braun EL, Ellis JD. 2016a. The complete mitochondrial genome of the Cape honey bee, *Apis mellifera capensis* Esch. (Insecta: hymenoptera: apidae). *Mitochondrial DNA Part B.* 1:817–819.
- Eimanifar A, Kimball RT, Braun EL, Ellis JD. 2016b. The complete mitochondrial genome of the hybrid honey bee, *Apis mellifera capensis* × *Apis mellifera scutellata*, from South Africa. *Mitochondrial DNA Part B.* 1:856–857.
- Eimanifar A, Kimball RT, Braun EL, Fuchs S, Grünwald B, Ellis JD. 2017a. The complete mitochondrial genome of *Apis mellifera meda* (Insecta: Hymenoptera: Apidae). *Mitochondrial DNA Part B.* 2:268–269.
- Eimanifar A, Kimball RT, Braun EL, Fuchs S, Grünwald B, Ellis JD. 2017b. The complete mitochondrial genome of an east African honey bee, *Apis mellifera monticola* Smith (Insecta: Hymenoptera: Apidae). *Mitochondrial DNA Part B.* 2:589–590.
- Eimanifar A, Kimball RT, Braun EL, Moustafa DM, Haddad N, Fuchs S, Grünwald B, Ellis JD. 2017c. The complete mitochondrial genome of the Egyptian honey bee, *Apis mellifera lamarkii* (Insecta: Hymenoptera: Apidae). *Mitochondrial DNA Part B.* 2:270–272.
- Gibson JD, Hunt GJ. 2015. The complete mitochondrial genome of the invasive Africanized Honey Bee, *Apis mellifera scutellata* (Insecta: Hymenoptera: Apidae). *Mitochondrial DNA.* 27:561–562.
- Haddad NJ. 2015. Mitochondrial genome of the Levant Region honeybee, *Apis mellifera syriaca* (Hymenoptera: Apidae). *Mitochondrial DNA.* 30:1–2.
- Haddad NJ, Adjlane N, Loucif-Ayad W, Dash A, Naganeeswaran S, Rajashekar B, Al-Nakkeeb K, Sicheritz-Ponten T. 2017. Mitochondrial genome of the North African Sahara Honeybee, *Apis mellifera sahariensis* (Hymenoptera: Apidae). *Mitochondrial DNA Part B.* 2:548–549.
- Hu P, Lu ZX, Haddad N, Noureddine A, Loucif-Ayad Q, Qang YZ, Zhao RB, Zhang AL, Guan X, Zhang HX, Niu H. 2015. Complete mitochondrial genome of the Algerian honeybee, *Apis mellifera intermissa* (Hymenoptera: Apidae). *Mitochondrial DNA.* 26:1–2.
- Jobb G, von Haeseler A, Strimmer K. 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evol Biol.* 4:18
- Takahashi J, Deowanish S, Okuyama H. 2017a. The complete mitochondrial genome of the dwarf honeybees, *Apis florea* and *Apis andreniformis* (Hymenoptera: Apidae) in Thailand. *Conserv Genet Res.* doi: [org/10.1007/s12686-017-0942-7](https://doi.org/10.1007/s12686-017-0942-7).
- Takahashi J, Hadisoelilo S, Okuyama H, Hepburn RH. 2017b. Analysis of the complete mitochondrial genome of *Apis nigrocincta* (Insecta: Hymenoptera: Apidae) on Sangihe Island in Indonesia. *Conserv Genet Res.* doi: [org/10.1007/s12686-017-0922-y](https://doi.org/10.1007/s12686-017-0922-y).
- Takahashi J, Rai J, Wakamiya T, Okuyama H. 2017c. Characterization of the complete mitochondrial genome of the giant black Himalayan honeybee (*Apis laboriosa*) from Nepal. *Conserv Genet Res.* doi: [10.1007/s12686-017-0765-6](https://doi.org/10.1007/s12686-017-0765-6).
- Takahashi J, Tingek S, Okuyama H. 2017d. The complete mitochondrial DNA sequence of endemic honeybee *Apis nuluensis* (Insecta: Hymenoptera: Apidae) inhabiting Mount Kinabalu in Sabah Province, Borneo Island. *Mitochondrial DNA Part B.* 2:585–586.
- Takahashi J, Wakamiya T, Kiyoshi T, Uchiyama H, Yajima S, Kimura K, Nomura T. 2016. The complete mitochondrial genome of the Japanese honeybee, *Apis cerana japonica* (Insecta: Hymenoptera: Apidae). *Mitochondrial DNA Part B.* 1:156–157.
- Tamura K, Stecher G, Peterson D, Filipiński A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol.* 30:2725–2729.
- Wakamiya T, Tingek S, Okuyama H, Kiyoshi T, Takahashi J. 2017. The complete mitochondrial genome of the cavity-nesting honeybee, *Apis koschevnikovi* (Insecta: Hymenoptera: Apidae). *Mitochondrial DNA Part B.* 2:24–25.