

Complete mitochondrial genome of the Japanese bumblebee, *Bombus hypocrita sapporensis* (Insecta: Hymenoptera: Apidae)

Jun-ichi Takahashi^a, Mana Nishimoto^a, Takeshi Wakamiya^a, Moe Takahashi^a, Takuya Kiyoshi^c, Koji Tsuchida^b and Tetsuro Nomura^a

^aDepartment of Life sciences, Kyoto Sangyo University, Kyoto, Japan; ^bFaculty of Applied Biological Sciences, Gifu University, Gifu, Japan;

^cDepartment of Zoology, National Museum of Nature and Science, Tokyo, Japan

ABSTRACT

In the present study, we describe the complete mitochondrial genome of the bumblebee, *Bombus hypocrita sapporensis* from the Rebus Island, in Hokkaido, Japan. The mitochondrial genome of *B. hypocrita sapporensis* includes a circular molecule of 15 700 bp. It contains 13 protein-coding genes, 22 tRNA genes, two rDNA genes and an A + T-rich control region. All protein-coding genes are initiated by ATA, ATG, and ATT codons and are terminated by the typical stop codon TAA or T, except for *ND4L*, which ends with TA. All tRNA genes typically form a cloverleaf secondary structure.

ARTICLE HISTORY

Received 4 February 2016
Revised 15 February 2016
Accepted 15 February 2016

KEYWORDS

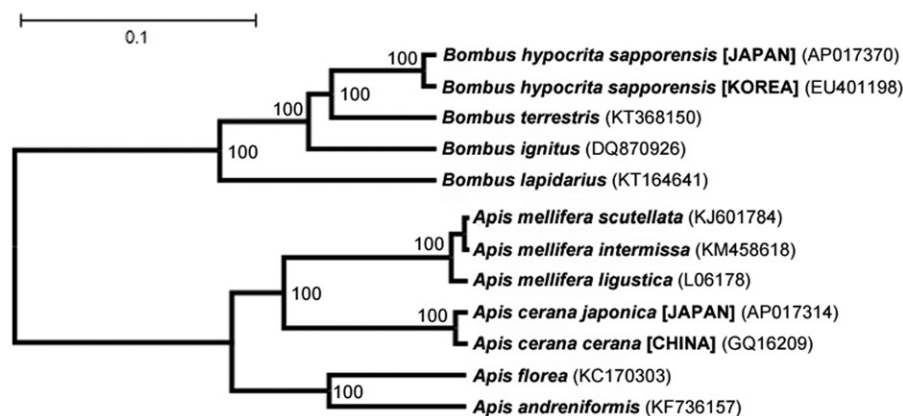
Asian bumblebee; *Bombus hypocrita*; genetic distance; identification

The Asian orange-tailed bumblebee, *Bombus hypocrita*, is a potential pollinator of greenhouse tomatoes that are distributed in Far East Asia. Mitochondrial DNA sequences of *B. hypocrita sapporensis* in South Korea have been analyzed except for the complete sequences of two genes (12S rDNA and tRNA-Gln) (Hong et al. 2008). This is the first study on the successful determination of the complete sequence of mitochondrial DNA of *B. hypocrita sapporensis* in Japan (accession number: AP017370).

An adult worker was collected from the Rebus Island in Hokkaido, Japan (Specimen is stored in the National Museum

of Nature and Science, Japan accession number: NSMT-I-HYM 73172). Genomic DNA isolated from the worker was sequenced using Illumina's HiSeq platform. The resultant reads were assembled and analyzed using the MITOS web server (Bernt et al. 2013, Germany) and GENETYX v.10 (GENETYX Corporation, Japan). Phylogenetic analysis was performed using the MEGA7 (Tamura et al. 2013) based on the nucleotide sequences of the 13 protein-coding genes.

The *B. hypocrita sapporensis* mitochondrial genome forms a 15 700 bp closed loop. This mitochondrial genome represents a typical hymenopteran mitochondrial genome and matches



with the *B. ignites* genome, in which it comprises 13 protein-coding genes, 22 putative tRNA genes, two rDNA genes and an A+T-rich control region (Cha et al. 2007; Du et al. 2015). The average AT content of the *B. hypocrita sapporensis* mitochondrial genome was 85.1%. Similar to the bumblebee mitochondrial genomes, the heavy strand (H-strand) encodes nine protein-coding genes and 14 tRNA genes, and the light strand (L-strand) encodes four protein-coding genes, eight tRNAs and two rDNA genes. The *ATP8* and *ATP6* genes shared 17 nucleotides, the *ATP6* and *COIII* genes shared one nucleotide, and the *ND6* and *Cytb* shared 11 nucleotides. Six protein-coding genes of the *B. hypocrita* mitochondrial genome started with ATA, the *ATP6*, *COIII*, *ND4* and *Cytb* genes with ATG, and the *ATP8* and *ND5* genes with ATT, all of which have been commonly found in the South Korean *B. hypocrita sapporensis* mitochondrial genome (Hong et al. 2008). The stop codon of each of these protein-coding genes was either TAA or T except for *ND4L* gene with TA, similar to the case in other bumblebees. All of the tRNA genes typically possessed cloverleaf secondary structures.

Nucleotide substitution rate between the *B. hypocrita sapporensis* mitochondrial genomes of Japan and South Korea was 99.0% (15 310/15 468). This corresponds well with the genetic distances generally between the *Apis cerana* mitochondrial genomes of Japan and China (Tan et al. 2011; Takahashi et al. 2016). Phylogenetic analysis was performed by applying 13 mitochondrial protein-coding genes with 12 closely related taxa (Figure 1). Classification of bumblebees is difficult because of the similarity between the female morphological traits of different species. In this study, phylogenetic analysis based on the mitochondrial DNA aided in the classification of the species of bumblebee. Complete sequence of the mitochondrial DNA in bumblebees is an effective information for species classification.

Disclosure statement

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

Funding information

This work was supported by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry (27013B) under Ministry of Agriculture, Forestry and Fisheries of Japan.

References

- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol Phyl Evol.* 69:313–319.
- Cha SY, Yoon HJ, Lee EM, Yoon MH, Hwang JS, Jin BR, Han YS, Kim I. 2007. The complete nucleotide sequence and gene organization of the mitochondrial genome of the bumblebee, *Bombus ignitus* (Hymenoptera: Apidae). *Gene.* 392:206–220.
- Du Q, Bi G, Zhao E, Yang J, Zhang Z, Liu G. 2015. Complete mitochondrial genome of *Bombus terrestris* (Hymenoptera: Apidae). *Mitochondrial DNA.* 5:1–2.
- Hong MY, Cha SY, Kim DY, Yoon HJ, Kim SR, Hwang JS, Kim KG, Han YS, Kim I. 2008. Presence of several tRNA-like sequences in the mitochondrial genome the bumblebee, *Bombus hypocrita sapporoensis* (Hymenoptera: Apidae). *Genes Genomics.* 30:307–318.
- 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.* [Epub ahead of print]. DOI:10.1080/23802359.2016.1144108.
- Tamura K, Stecher G, Peterson D, Filipksi A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol.* 30:2725–2729.
- Tan HW, Liu GH, Dong X, Lin RQ, Song HQ, Huang SY, Yuan ZG, Zhao GH, Zhu XQ. 2011. The complete mitochondrial genome of the Asiatic cavity-nesting honeybee *Apis cerana* (Hymenoptera: Apidae). *PLoS One.* 6:e23008.