

## The complete mitochondrial genome of the bumblebee, *Bombus hypocrita sapporensis* (Insecta: Hymenoptera: Apidae) from Hokkaido Island, Japan

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### ABSTRACT

The complete mitochondrial genome of the Japanese bumblebee *Bombus hypocrita sapporensis* from Hokkaido Island, Japan is analysed using next generation sequencing. The mitochondrial genome of *B. h. sapporensis* was observed to be a circular molecule of 15,835 bp. The average AT content in the *B. h. sapporensis* mitochondrial genome was 85.53%. It was predicted to contain 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes, along with one A+T-rich control region. The PCGs had ATA, ATG, or ATT as the initiation codon and were terminated by the typical stop codon TAA, except for *Cytb*. All the tRNA genes typically formed a cloverleaf secondary structure, except for *trnE*, *trnF*, and *trnS1*. The molecular phylogenetic analysis indicated that the *B. h. sapporensis* from Hokkaido Island population was most similar to that of the geographically isolated *B. h. sapporensis* from Rebun Island.

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Bumblebee; Illumina sequencing; subspecies; *Bombus hypocrita*; Hokkaido

The orange-tailed bumblebee in Far East Asian continent including North China was *Bombus patagiatus*, widely distributed to Russia, on the other hand, the orange-tailed bumblebee in Japan was true *Bombus hypocrita* (Williams, An, et al. 2012; Williams, Brown, et al. 2012). Williams, An, et al. (2012) and Williams, Brown, et al. (2012) indicated that orange-tailed bumblebee was a separate species in Japan and North China in mitochondrial DNA *COI* gene analyses using the DNA barcoding method. In morphological taxonomic analysis, Japanese *B. hypocrita* is classified into two subspecies, *B. hypocrita sapporensis* in Hokkaido Island, and *B. h. hypocrita* in Honshu, Shikoku and Kyushu Islands (Sakagami and Ishikawa 1969; Ito 1998; Ito and Munakata 1979), but the phylogenetic relationship of two *B. hypocrita* subspecies on Japan remains uncertain (Takahashi, Nishimoto, et al. 2016; Nishimoto et al. 2017; Takeuchi et al. 2017). Here we analysed the complete mitochondrial genome of orange-tailed bumblebee *B. h. sapporensis* from Hokkaido Island in Japan.

Adult workers of the *B. h. sapporensis* on wild flowers in the Otofuke Town (42°56'43"N 143°12'06"E), Hokkaido Islands, Japan, were collected in July 2017. The collected workers were transferred immediately to 99% ethanol for mitochondrial DNA analysis (the specimen was stored in the National Museum of Nature and Science, Japan, accession number: NSMT-I-HYM 74241). Genomic DNA isolated from males was sequenced using Illumina's MiSeq platform (Illumina, San Diego, CA). The resultant reads were assembled and annotated using the MITOS web server (Bernt et al. 2013) and Geneious

R9 (Biomatters, Auckland, New Zealand). Phylogenetic analysis was performed using the TREEFINDER (Jobb 2015) based on nucleotide sequences of the 13 protein-coding genes (PCGs).

The average AT content of the *B. h. sapporensis* mitochondrial genome was 85.53%. Similar to the *B. hypocrita* mitochondrial genomes from Honshu and Rebun Islands (Takahashi, Nishimoto, et al. 2016; Nishimoto et al. 2017), the heavy strand (H-strand) was predicted to contain nine PCGs and 13 tRNA genes, and the light strand (L-strand) was predicted to contain PCGs, nine tRNA genes, and two rDNA genes (Figure 1). The genes, *ATP8* and *ATP6*, shared 19 nucleotides, *ND4* and *ND4L* shared one nucleotide, and *ND6* and *Cytb* shared 13 nucleotide. Six PCGs of the *B. h. sapporensis* mitochondrial genome started with ATA, *ATP6*, *COIII*, *ND4*, and *Cytb* started with ATG, and *COII*, *ND5* and *ND4L* started with ATT; these starting codons have been found to be common in the other *Bombus* mitochondrial genomes (Cha et al. 2007; Hong et al. 2008; Du et al. 2015; Takahashi, Sasaki, et al. 2017; Zhao, Huang, et al. 2017; Zhao, Wu, et al. 2017). The stop codon in each of these PCGs was TAA, except for *Cytb*, which had TAG, as in other bumblebees.

Molecular phylogenetic analysis was constructed by applying 13 mitochondrial PCGs with 12 closely related taxa (Figure 2). The genetic distance and mutation site of the *B. hypocrita* in Japan taxa mitochondrial genomes were 0.0002–0.0018 and 2–20, on the other hand that of the orange-tailed bumblebee in Asia continent and the Japanese *B. hypocrita* were 0.0103–0.0107 and 113–117. The *B. hypocrita* from three Japanese Islands were shown to



be a valid subspecies from the *B. hypocrita* in Asia continent by complete mitochondrial DNA sequence.

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## Disclosure statement

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

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