



SHORT NOTE

Characterizing the Mitogenome of the Endemic Bumblebee Subspecies from the Canary Islands for Conservation Purposes

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Abstract

The taxonomic status of *Bombus terrestris* subspecies is complex and has deep implications in the management of commercial bumblebees for crop pollination as well as in the establishment of appropriate conservation plans. Herein, the complete mitogenome of the endemic Canary Islands subspecies *Bombus terrestris canariensis* is newly sequenced and compared with available mitochondrial sequences in order to shed light into its taxonomic status. The obtained sequence of the mitochondrial genome was 17,300 bp in length and contained 37 genes, including 13 protein-coding genes (PCGs), two rRNAs, and 22 tRNAs and a partial sequence of the AT rich control region. The phylogenetic analysis of PCGs of the mitogenome was congruent with its subspecies status and a close relationship with the North African subspecies *africanus* as previously suggested. The sequencing of the mitogenome of *B. t. canariensis* provides useful genetic information to study the conservation genetics and genetic diversity of these island bumblebee populations.

Bombus terrestris is the most abundant and widespread bumblebee in the West Palearctic where it has differentiated into eight subspecies that differ in morphology and genetic characters, behaviour and phenological traits (Rasmont et al., 2008). This species has a great economic and ecological importance because several *B. terrestris* subspecies have been massively reared and exported worldwide for crop pollination (Velthuis & van Doorn, 2004). This commercialization has resulted in reproductive interference with native species (Tsuchida et al., 2019) or hybridization with native subspecies (Cejas et al., 2018, 2020; Seabra et al., 2019; Bartomeus et al., 2020), multiple invasions with competitive displacement of native taxa (Matsumura et al., 2004; Ings et al., 2006; Inoue et al., 2008; Nagamitsu et al., 2010; Morales et al., 2013) and possibly pathogen spillover to local bumblebees (Goka et al., 2001; Arbetman et al., 2013; Schmid-Hempel et al., 2014).

Given these problems, the trade of the different commercial *B. terrestris* subspecies has been restricted in few places such as the Israel, Norway, Turkey, the United Kingdom and the Canary Islands, where only the local subspecies *B. terrestris canariensis* is allowed to be commercialized (Velthuis & van Doorn, 2006; Lecocq et al., 2016).

Molecular, morphological and pheromone markers have been used to clarify the taxonomy of the *terrestris* subspecies complex. In particular, the endemic taxon of the Canary Island was firstly described as a *B. terrestris* subspecies (Perez, 1895) and later elevated to species status by Erlandsson (1979) based on its distinct coloration pattern and geographic isolation. Several mtDNA and nuclear markers have supported both species (Widmer, 1998) and subspecies status (Estoup et al., 1996) or remain unclear (Bertsch, 2010). Integrative taxonomic approaches combining independent



markers such as pheromones, morphological and molecular data generated contrasting results supporting both species (De Meulemeester, 2012) and subspecies status (Lecocq et al., 2016). Furthermore, crossing experiments showed that *canariensis* interbreed with other *B. terrestris* subspecies leading to fertile offspring (van den Eijnde & de Ruijter, 2000), what has been used as an evidence of its subspecies status (Velthuis & van Doorn, 2006), although interspecific mating cannot be rule out (Kondo et al., 2009; Yoon et al., 2009).

The taxonomy of *B. terrestris* subspecies has deep implications also in the establishment of appropriate conservation plans (Lecocq et al., 2016). Therefore, a solid taxonomic status must be achieved using novel molecular approaches. Mitogenome sequence combined with other molecular markers has successfully resolved phylogenetic and taxonomic issues in several taxa, resulting in an effective tool for bee phylogeny and conservation genetics (Nishimoto et al., 2018; Du et al., 2016; Lin et al., 2019). Here we have sequenced the complete mitogenome of the *canariensis* taxon and compared it with available mitochondrial sequences to shed light into its taxonomic status.

To ensure an adequate amount of mitochondrial DNA for sequencing (Cejas et al., 2020), muscle tissue was dissected from each of ten *B. t. canariensis* individuals collected from flowers at a single locality on La Gomera (Canary Islands,

Spain) and pooled. DNA extraction and mtDNA enrichment was done using the miniprep kit of Qiagen (Hilden, Germany). Sequencing was performed on a HiSeq2000 (Illumina) using an Illumina TruSeq Nano DNA Library Prep Kit for 350 base pairs (bp) (Macrogen, South Korea). An Agilent Technologies 2100 Bioanalyzer with a DNA 1000 chip was used to measure the size of the raw library reads. Reads were filtered with the software FastQC (Babraham Bioinformatics 2012) before assembling them into contigs and scaffolds as in Cejas et al. (2020). Annotation of PCGs, transfer RNA (tRNA) genes and ribosomal RNA (rRNA) genes for the consensus sequence was obtained by comparison with previous annotated genomes in the web server MITOS (Bernt et al., 2013). In order to resolve the phylogenetic position of *canariensis*, ten mitogenomes of related species and subspecies were obtained from GenBank (Supplementary Material 1). Two mitochondrial markers (*cox1* and *cytb*) comprising 1,307 bp were used for all the subspecies with no mitogenome available (except for *africanus* with only sequence data of *cox1*). The phylogenetic position of *canariensis* was estimated from a concatenated dataset including the 13 protein coding genes (PCGs) using the software MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003). The best-fitting substitution model was assessed with IQ-TREE. A phylogenetic tree was constructed using *B. consobrinus* (MF995069) as outgroup.

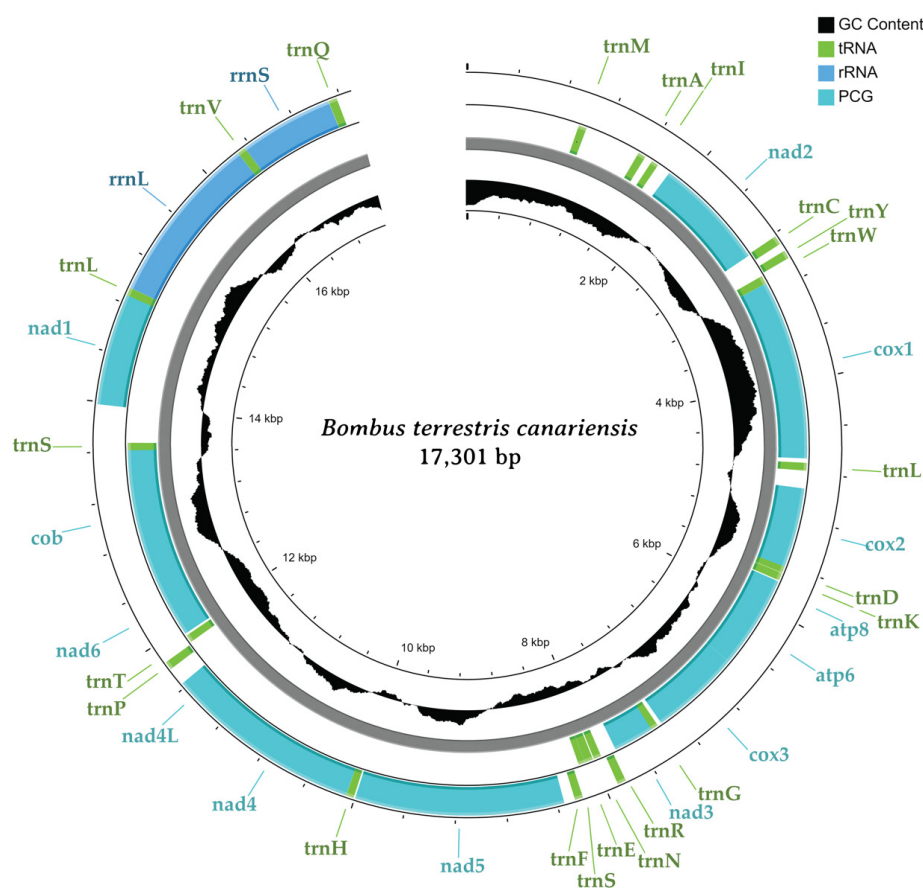


Fig 1. Physical map of the obtained sequence mitochondrial genome of *Bombus terrestris canariensis* in absence of the complete A+T control region. Protein coding genes (PCG) are indicated in light blue, tRNA genes in green, and rRNA genes in dark blue. The GC content is indicated in black.

The A+T control region was not completely sequenced, owing to its extreme variability and the pooling approach followed. Thus, the mitogenome of *B. t. canariensis*, was sequenced to a length of 17,300 bp (GenBank accession number MW959771) (Fig 1). The sequenced part varies in length in comparison to other *B. terrestris* subspecies (*B. t. terrestris*: 17,232 bp; *B. t. lusitanicus* 17,049 pb) mainly due to the presence of indels in intergenic regions. Gene order was consistent with published data (Cejas et al., 2020). It contained 13 protein-coding genes (PCGs), two rRNAs, and 22 tRNAs and a partial sequence of the AT rich control

region. The average A+T content was 86.4%, slightly higher than that in other *B. terrestris* subspecies (*B. t. lusitanicus* and *B. t. terrestris* 86%). This A+T content was higher in non PCGs (87.5%) than in the PCGs region (83.7%). Most of the variation observed in the available mitogenomes of *terrestris* subspecies occurred in non PCGs. PCGs of *B. t. canariensis* showed 193 SNPs, especially in *NAD4* (34), *NAD5* (33) and *cox1* (27) genes. Transitions (83%) were more frequent than transversions in the SNPs. No indels were found within the PCGs whereas a 6 bp deletion was observed in the large RNA gene sequence.



Fig 2. Bayesian phylogenetic tree showing the relationship between *Bombus terrestris canariensis* (in red) and other eight *B. terrestris* subspecies. An arrow indicates the *B. t. canariensis* mitogenome sequenced in this study and bold names the mitogenomes used. Empty and filled circles indicates posterior probabilities of 0.90-0.95 and >0.95 respectively.

The combined analysis of concatenated PCGs of mitogenomes and available markers of the different subspecies showed high support for all the basal nodes and low support for the nodes within *terrestris* clade (Fig 2). *B. terrestris* appeared within the subgenus *Bombus*, related with species such as *B. lucorum*, *B. hypocrita* and *B. cryptarum*. All the studied *terrestris* subspecies including *canariensis* appeared as monophyletic in a supported clade, thus reinforcing the subspecies status of *canariensis*. However, other taxon such as *xanthopus* that has been previously elevated to species (Lecocq et al., 2015) appeared within the *B. terrestris* clade.

These results should be taken with caution as only three mitogenomes were available for the *terrestris* subspecies and only partial mitochondrial data (1,307 bp) have been analysed for the remaining subspecies. Given its importance as a commercial species, further mitogenomes of the remaining subspecies will establish a solid taxonomy of the group. The sequencing of the *B. t. canariensis* genome itself provides additional genetic information useful for studying the conservation genetics of these island bumblebee populations, creating a framework for establishing conservation programs for pollination networks of *B. terrestris* subspecies with locally endemic flora.

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Authors' Contribution

All the authors have given their consent to participate in the redaction of the manuscript. CR, IM and PDIR conceived the ideas and designed the experiments. DC did the DNA extractions and performed the assembly of the mitogenome. CR performed most of the analyses with the assistance of DC. CR and PDIR wrote the manuscript with input from IM. All the authors critically reviewed the manuscript.

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Electronic Supplementary Material

Supplementary file 1: Sampling information of the individuals used for the phylogenetic analysis.

