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 (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.
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.](image-url)
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 (Lecoq 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.