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The nuclear and mitochondrial genomes of the bellicose bumblebee (*Bombus bellicosus*, Hymenoptera: Apidae), a threatened pollinator in a changing South American landscape

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Abstract

We present the first genome of a Brazilian bumblebee species, the bellicose bumblebee (*Bombus bellicosus*). This is an endemic species in southern South America facing local extinction due to habitat loss and climate change. During the COVID-19 social distancing in Brazil, we launched a citizen science initiative via social media to locate remaining bellicose bumblebee populations, leading to the collection of a specimen for genome sequencing. Analysis of the novel genome revealed lower genetic diversity in the bellicose bumblebee compared to a widespread related species (*Bombus pascuorum*). However, the absence of extensive runs of homozygosity indicated a lack of recent inbreeding, offering a promising perspective for the conservation of this species. Furthermore, demographic history analysis indicates population expansion during past glacial periods, in contrast to Palearctic bumblebees that suffered a stark decline during glaciations. Our findings provide invaluable information for the conservation of this species and for further studies about its biology and evolution, particularly under a scenario of rapid environmental change.

Key words: Apidae, citizen science, endangered species, genome assembly, heterozygosity, Hymenoptera, runs of homozygosity

Introduction

Genomes are crucial for conservation efforts as they provide insights into the genetic diversity and adaptability of species, facilitating the development of targeted conservation strategies, population monitoring, genetic rescue, and threat identification (Formenti et al. 2022). For bees, genomic knowledge helps identify and preserve traits important for pollination, fundamental for ecosystem health and sustainable agriculture. Significant progress has been made through initiatives such as the Darwin Tree of Life project (The Darwin Tree of Life Project Consortium 2022) and Beenome100 (https://www.beenome100.org/), focusing on the regional fauna of the UK and USA. Neotropical bee species, by contrast, remain underrepresented in genomic databases. This disparity is particularly evident among bumblebees (*Bombus*), where only one of the 31 Neotropical species has been sequenced to date—a milestone achieved recently (Martínez et al. 2024; as of January 2025). Such gaps hinder our understanding of bumblebee biology and evolution, impeding conservation efforts and pollinator management policies. This lack of data is critical for threatened species such as *Bombus bellicosus*, highlighting the urgent need for genomic resources.

The bellicose bumblebee (*B. bellicosus*) is poorly understood, with much of its biology unknown. Historically found in open grasslands from northwestern Argentina to the state of Paraná in Southern Brazil (Moure and Sakagami 1962), its distribution has shrunk drastically (Fig. 1), primarily due to habitat loss and pesticide use (further discussed in Martins and Melo 2010). The last specimens from Paraná were collected in the early 1990s (Bortoli and Laroca 1990), and subsequent surveys failed to find it in its northern range, suggesting local extinction (Fig. 1; Gonçalves and Melo 2005). The bellicose bumblebee has a unique adaptation among Neotropical bumblebees: a wax layer covering the brood chamber, which is typical of temperate bumblebee species (Varela 1992*a*, 1992*b*). This wax layer enables the colony to maintain higher

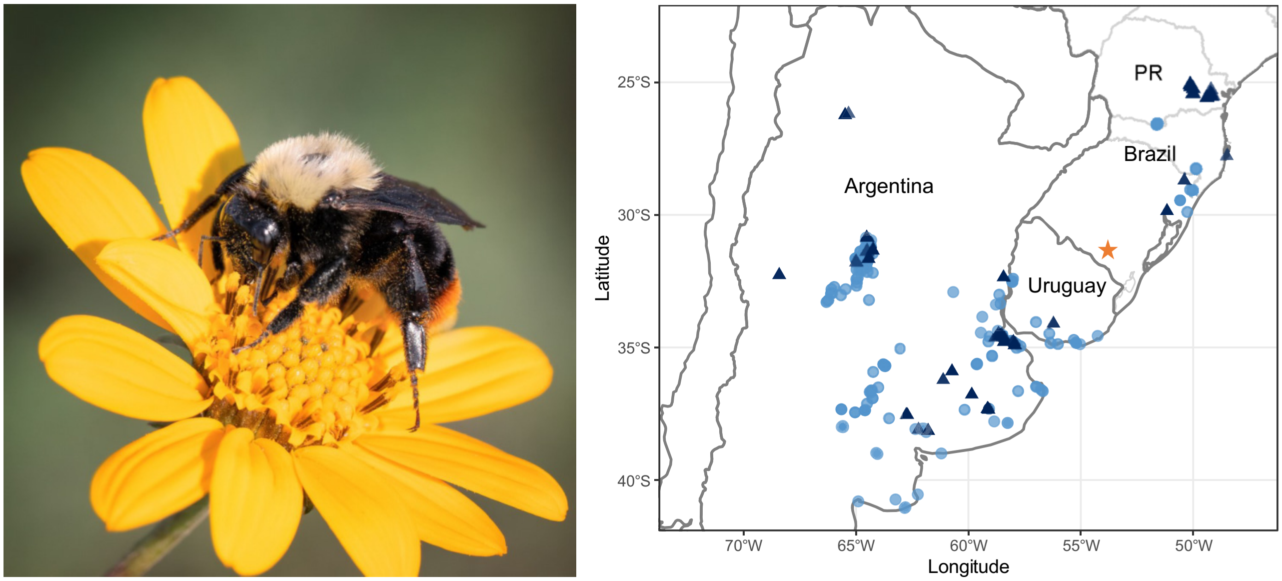


Fig. 1. Photograph and distribution records of the bellicose bumblebee (*Bombus bellicosus*) in southern South America. Brazilian state boundaries are shown on the map. The orange star indicates the specimen collected for sequencing. Dark blue triangles indicate specimens collected before 1990, and light blue dots indicate specimens collected after 1990. Populations in the northern range of this species' occurrence, in the Brazilian state of Paraná (PR), are considered locally extinct (Martins and Melo 2010). Map produced using functions and shapefiles of R package geobr (Pereira and Gonçalves 2019) in R v4.3.2 (R Core Team 2023). Occurrence data sourced from GBIF (https://www.gbif.org/), considering museum specimens and iNaturalist observations (https://www.inaturalist.org/). Image courtesy of Gabriel Paladino Ibáñez.

nest temperatures during cold seasons. However, this adaptation may become detrimental under future warmer climates (Martins et al. 2015; Krechemer and Marchioro 2020), posing a significant threat to the species.

Although potentially extinct at the northern edge of its range (Martins and Melo 2010), the conservation status of the bellicose bumblebee has not been formally assessed. Climate change and land use are increasing the pressure on this species (Martins et al. 2015), together with the imminent threat posed by the invasive buff-tailed bumblebee (*Bombus terrestris*) to native South American bumblebee populations, as seen with *Bombus dahlbomii* in Patagonia (Morales et al. 2013; Fontúrbel et al. 2021). Ecological niche models show that the entire range of *B. bellicosus* is highly suitable for *B. terrestris* invasion and is also connected to known invasion fronts (Acosta et al. 2016). The arrival of *B. terrestris* would not only intensify competition for resources but also raise the risk of pathogen spillover, as *B. terrestris* has been associated with the transmission of parasites and diseases to native bees and managed honeybees (Plischuk et al. 2011; Arbetman et al. 2013; Morales et al. 2013; Schmid-Hempel et al. 2014; Aizen et al. 2019). In this context, developing genomic resources is crucial for assessing genetic diversity, monitoring population resilience, and informing conservation strategies to prevent extinction.

Here, we present the first genome of a Brazilian bumblebee species, the bellicose bumblebee (*B. bellicosus*). The specimen for sequencing was found through a citizen science project in Brazil during COVID-19 social distancing in 2021. We used this genome to gain insights into the demographic history of the bellicose bumblebee with a focus on conservation genomics. We also compared this genome with data from two other widespread bumblebee species: the common carder bee (*Bombus pascuorum*), a close relative, and the commercially managed buff-tailed bumblebee. Given that the bellicose bumblebee is a threatened species, we tested two hypotheses: (1) heterozygosity levels, as a proxy for genetic diversity, will be lower in the bellicose bumblebee than in the two more widespread species; (2) large runs of homozygosity (ROH), suggesting inbreeding, will be present in the bellicose bumblebee genome. The addition of this Neotropical bumblebee genome broadens the scope of global efforts to study bumblebee genetic diversity and evolutionary history, addressing gaps in knowledge about pollinators from tropical regions. Our findings provide a valuable resource for future conservation efforts and the development of public policies aimed at preserving and managing pollinators, particularly in South America, where such initiatives are still in the early stages.

Materials and methods

Wanted: finding the bellicose bumblebee

Bombus bellicosus is a relatively rare species in southern Brazil, with greater abundance observed in Argentina and Uruguay (Fig. 1). Our aim was to obtain a fresh specimen for genomic sequencing; however, our project started just a month before the COVID-19 lockdown in Brazil. Consequently, fieldwork became unfeasible. To overcome this obstacle, we initiated a citizen science campaign via social media in 2021, asking people to share photographs of bellicose bumblebees observed in their surroundings (Fig. S1). This campaign garnered significant engagement, and resulted in approximately 80 participants submitting images within a 2-month timeframe, claiming sightings of the bellicose bumblebee. Most submissions featured much more common bumblebee species (such as *Bombus pauloensis* and *Bombus morio*), or the large carpenter bees (*Xylocopa*) that share the vernacular name "mamangava" with bumblebees in Brazil. This difficulty in tracking down the bellicose bumblebee highlights its rarity and the decline it has been suffering. However, our partnership with the community eventually made it possible to find a specimen for sequencing.

Sampling, DNA extraction, and sequencing

We generated genomic DNA from the leg muscle of a single female worker collected at Hulha Negra, RS, Brazil (31°29′18.8″S, 53°49′55.1″W; Fig. 1) in January 2022. Sampling was undertaken using SISBIO scientific permit number 81086-2. Morphological specimen identification followed the key provided in Françoso et al. (2016*a*). The specimen used for sequencing was deposited at the frozen collection of the *Drosophila* Lab of the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). DNA quality was estimated using a Nanodrop DN-1000 Spectrophotometer (Thermo Fisher), and DNA concentration was assessed using a Qubit Fluorometer (Thermo Fisher). We sent the genomic DNA to Macrogen (Seoul, South Korea) for library construction and sequencing. TruSeq Nano 350 bp libraries were generated and sequenced on an Illumina NovaSeq platform to a depth of 150x coverage, assuming a genome size around 250 Mbp (Sun et al. 2021).

Genome assembly and scaffolding

Quality parameters for the raw data were assessed using FASTQC v0.119 (Andrews 2010). Subsequently, adapter sequences were removed from the reads employing Trimmomatic v0.36 (Bolger et al. 2014). Expected genome size was estimated with GenomeScope v2.0 (Ranallo-Benavidez et al. 2020) using as input the k-mer frequency analysis performed by Meryl v1.3 (Rhie et al. 2020). The trimmed reads were subjected to assembly utilizing SPAdes v3.15.3 (Prjibelski et al. 2020), with the "–careful" option to minimize the occurrence of mismatches. Assembly statistics were obtained with QUAST v5.2.0 (Mikheenko et al. 2018). We aligned the contigs against a reference genome sequence from the closely related species *B. pascuorum* (Crowley et al. 2023*a*; GCF_905332965.1) using RagTag v2.1.0 (Alonge et al. 2022). Finally, the assembly was polished using pilon v1.20 (Walker et al. 2014). Assembly completeness was evaluated using two approaches. First, we assessed the presence of conserved single-copy orthologs by analyzing the Hymenoptera gene set ($n = 5991$) from BUSCO v5.4.7 (Manni et al. 2021) during different steps of our assembly workflow. Second, we used Merqury v1.1 (Rhie et al. 2020) to evaluate completeness and quality of the raw assembly obtained with SPAdes.

Mitochondrial genome assembly and annotation were conducted using MitoFinder v1.4.1 (Allio et al. 2020), using as annotation references the NCBI RefSeq mitochondrial genomes of *Bombus waltoni* (NC_045283), *Bombus hypocrita sapporensis* (NC_011923), *B. terrestris lusitanicus* (NC_045178), and *B. terrestris terrestris* (NC_045179). Annotations provided by MitoFinder were inspected manually and cross-checked with ARWEN (Laslett and Canbäck 2008) and MITOS2 WebServer (Bernt et al. 2013).

Nuclear genome annotation

A repeat library was constructed with RepeatModeler v2.0.5 (Flynn et al. 2020) and genome softmasking was performed with RepeatMasker v4.1.2 (Smit et al. 2013) using the generated custom library. The softmasked genome was used as input for BRAKER v3.0.8 (Gabriel et al. 2024) to automatically train and predict gene annotations. Given that no RNASeq dataset is available for *B. bellicosus*, we implemented the BRAKER3 pipeline C, which predicts genes with AUGUSTUS (Keller et al. 2011) and GeneMark-ETP (Brůna et al. 2024), setting as evidence the OrthoDB v11 arthropod dataset (Kuznetsov et al. 2023) and the proteomes of *B. terrestris* (Crowley et al. 2023*b*) and *B. pascuorum* (Crowley et al. 2023*a*). Output was passed to eggNOG-mapper v2.1.8 (Huerta-Cepas et al. 2017) and InterProScan v5.59 (Jones et al. 2014) for functional annotation. Quality of the annotation was again assessed with BUSCO as previously described, but in the annotated gene set (protein) mode.

Genotyping

The following analyses were conducted for the pseudochromosomes of the bellicose bumblebee, as well as for the chromosome-level genomes of *B. terrestris* (Crowley et al. 2023*b*; GCF_910591885.1) and *B. pascuorum* (Crowley et al. 2023*a*; GCF_905332965.1). We chose these two species because *B. terrestris* is a model species among bumblebees due to its commercial use, whereas *B. pascuorum* belongs to the same subgenus of *B. bellicosus* (*Thoracobombus*) and thus is the closest species with a high-quality genome available. Both species are from the Palearctic region. Downstream analyses were restricted to the pseudochromosomal regions to maintain result comparability due to the high number of contigs obtained for *B. bellicosus* (see "Results and Discussion" section).

Raw reads were trimmed with the same parameters described above. The trimmed reads were mapped against the assembled genome for each species using BWA-MEM v0.7.17 (Li 2013). Then, we used Samtools to sort reads and fix read pair information before adding read group tags with Picard v3.1.1. Next, we removed duplicates with Picard and used the Genome Analysis Toolkit (GATK v.3.8; McKenna et al. 2010) to conduct local realignment around indels (RealignerTargetCreator and IndelRealigner) and genotype calling (HaplotypeCaller followed by GenotypeGVCFs). Both variant and invariant genotypes were converted to variant call format (VCF) files using -ERC BP_RESOLUTION -out_mode EMIT_ALL_SITES options with HaplotypeCaller and -allSites -stand_call_conf 0 options with GenotypeGVCFs.

The VCF files were filtered to mask potentially erroneous genotype calls using GATK's VariantFiltration recommended filters: QD < 2.0, FS > 60.0, MQ < 40.0, MQRankSum < -12.5, $8.0 <$ ReadPosRankSum < -8.0, SOR > 4.0. Then, we used BCFtools (Danecek et al. 2021) to maintain only biallelic sites and exclude sites with either insufficient or excessive read depth (1/3X and 2X the genome-wide average, respectively).

Heterozygosity and runs of homozygosity

As a proxy of genetic diversity, we evaluated genome-wide heterozygosity for *B. bellicosus*, *B. pascuorum*, and *B. terrestris*. Heterozygosity per genome was calculated from the filtered VCF files using pixy (Korunes and Samuk 2021), by estimating the proportion of heterozygous sites in non-overlapping sliding windows of 10 kbp across the genome while taking missing sites into account. Subsequently, we estimated ROH by counting contiguous windows identified by pixy exhibiting an average heterozygosity below 0.05% using a custom R script (https://github.com/leonardotgoncalves/bellicosus/blob/main/ROH.R). We restricted our analysis to ROH where the number of sites represented at least half of the window size.

Population size history (PSMC)

We employed the pairwise sequentially Markovian coalescent model (PSMC, https://github.com/lh3/psmc) to gain insights into past demographic events of the bellicose bumblebee. PSMC uses whole genome data from single individual diploid samples to estimate loci coalescence rates and track effective population size (N_e) history (Li and Durbin 2011). This analysis is useful for identifying demographic bottlenecks, population expansions, and shifts in effective population size, all of which can provide insights into how past evolutionary events may have shaped current population structure. A PSMC was built using the default parameters, and to evaluate variance in N_e we conducted 100 bootstraps. Time on PSMC plots was rescaled using a 1-year generation time and a mutation rate of 3.6×10^{-9} per site per year, estimated from *B. terrestris* (Liu et al. 2017).

Results and discussion

Genome overview

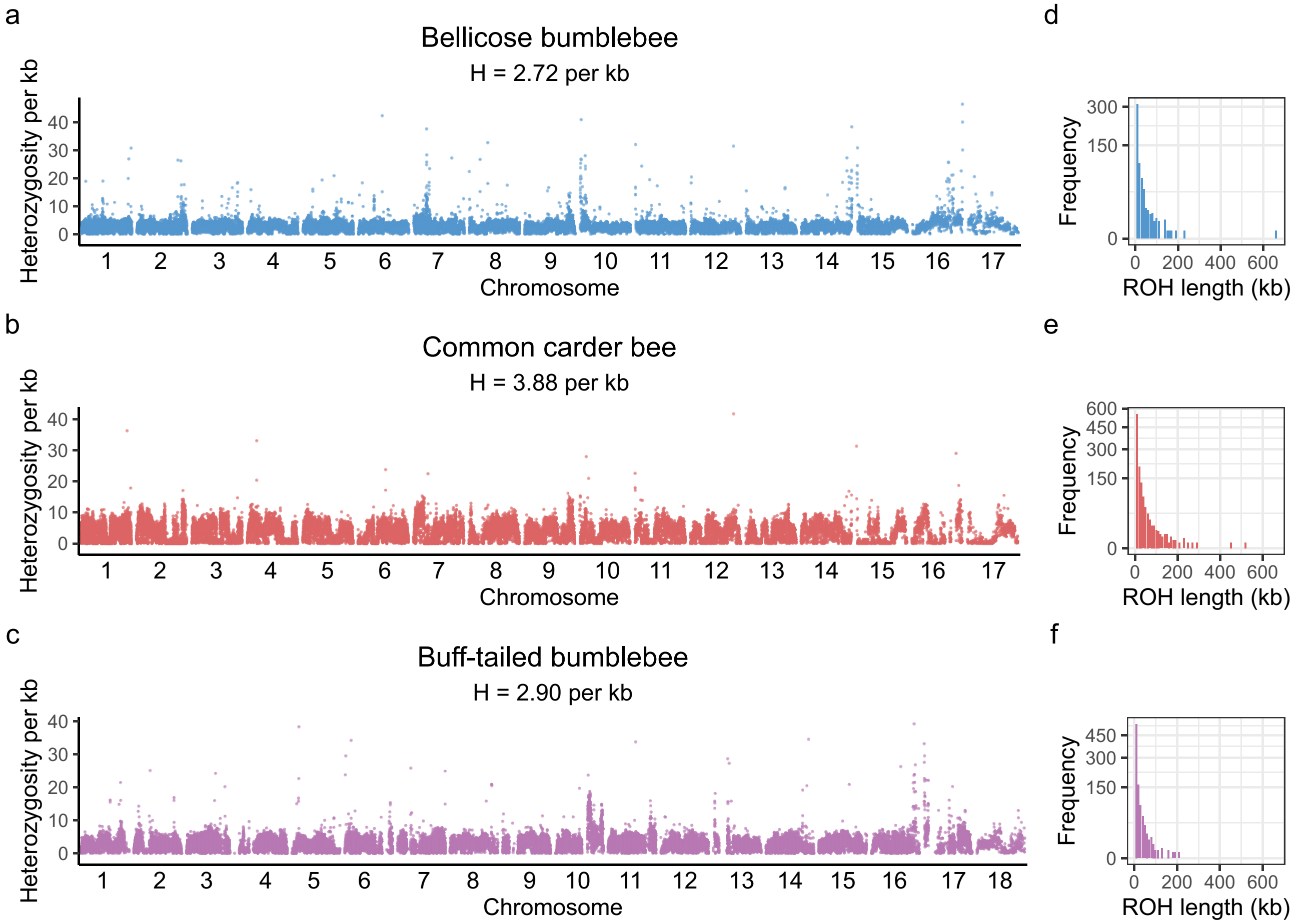
The assembled genome of the bellicose bumblebee consisted of 76 466 contigs with an N50 of 18 032 bp and an L50 of 5942, totaling 462 573 696 bp, and the longest contig spanning 527 306 bp (see Table S1 for details). GenomeScope analysis using k-mers estimated the genome size to be approximately 333 Mbp (k-mer = 21, k-cov = 46), with an average heterozygosity of 0.33% (Fig. S2). These estimates are consistent with previously reported genome sizes for other bumblebee species, which range from 230 to 393 Mbp (Sun et al. 2021; Crowley et al. 2023*b*), as well as with heterozygosity levels (e.g., 0.33% for *B. terricola*, Kent et al. 2018). The initial assembly demonstrated high base accuracy, as indicated by a Merqury quality value of 47.2. BUSCO analyses revealed a high level of completeness, with 94.4% of conserved hymenopteran orthologs identified as complete (83.7% single-copy, 10.7% duplicated, 2.6% fragmented, 3.0% missing; Table S3).

To improve assembly quality, scaffolding with RagTag produced 17 chromosomal pseudomolecules, covering 84.2% of the chromosomes of the common carder bee reference genome (Table S2), which was assembled using long reads and chromatin conformation data (Crowley et al. 2023*a*). This process improved assembly completeness, as reflected in BUSCO analyses: complete orthologs increased to 97.3%, while fragmented orthologs decreased (86.7% single-copy, 10.6% duplicated, 0.8% fragmented, 1.9% missing; Table S3). Focusing exclusively on the 17 pseudochromosomes reduced duplicated orthologs (0.2%) while maintaining a high percentage of single-copy orthologs (96.1%) and low levels of fragmented (1.0%) and missing orthologs (2.7%; Table S3). These results are comparable to other high-quality reference bee genomes, such as *B. terrestris* (96.5%; Crowley et al. 2023*b*), *Apis mellifera* (97.7%; Wallberg et al. 2019), and *Melipona bicolor* (96.3%; Araujo et al. 2024), also surpassing the average for hymenopteran genomes (94.0%; Hotaling et al. 2021).

Repetitive elements comprised 11.5% of the 17 pseudochromosomes in the bellicose bumblebee genome, a proportion consistent with other bumblebee species (ranging from 9.7% to 17.9%; Sun et al. 2021), which is lower than the average observed in most insects (Sun et al. 2023). When unplaced contigs were analyzed separately, repetitive elements accounted for 15.2% of their base pairs. Combining both the pseudochromosomes and unplaced contigs, the overall repetitive element content was 13.3%, with unclassified repeats constituting 3.6%, and Class I and Class II transposable elements making up 1.6% and 0.9%, respectively. The majority of identified repeats in both pseudochromosomes and unplaced contigs remained unclassified (7.5% and 8.4%, respectively). Further details and statistics on repetitive element annotations are provided in Table S4. Our findings underscore the challenges associated with repetitive element classification in non-model insects using current automated pipelines (Sproul et al. 2023). Future investigations employing long-read sequencing technologies and genome editing techniques will enhance transposable element identification in *B. bellicosus* and other bumblebees, also helping to elucidate mechanisms underlying their evolutionary dynamics and adaptive strategies (Sun et al. 2023).

Using the BRAKER workflow, we predicted 15 999 protein-coding genes and 18 044 transcripts (Data S1) on the 17 pseudochromosomes, encompassing 96.2% of hymenopteran BUSCO proteins (83.0% single-copy, 12.2% duplicated; Table S3). These results agree with gene counts reported for other bumblebee genomes, regardless of sequencing approaches used, such as short reads (Sun et al. 2021), long reads (Koch et al. 2024), or hybrid methods (Crowley et al. 2021, 2023*a*, 2023*b*). On unplaced contigs, BRAKER identified 22 982 protein-coding genes and 24 602 transcripts (Data S2), which included 46.4% of the hymenopteran BUSCO proteins (42.7% single-copy, 3.7% duplicated; Table S3). Many of these are likely from duplicated or heterozygous regions that could not be assembled into chromosomal scaffolds, although some may also originate from contamination.

Fig. 2. (*a*–*c*) Distribution of genome-wide heterozygosity in the bellicose bumblebee (*Bombus bellicosus*), common carder bee (*Bombus pascuorum*), and buff-tailed bumblebee (*Bombus terrestris*). Each dot represents the mean heterozygosity across non-overlapping 10 kbp windows of bumblebee chromosomes. *H* denotes mean heterozygosity across the entire genome. (*d*–*f*) Frequency and size of runs of homozygosity (ROH) across bumblebee genomes. A ROH is a contiguous fragment of a chromosome with negligible heterozygosis (here, <0.0005 per site). Bars represent the square root of the frequencies, while values on the *y*-axis indicate the original counts.



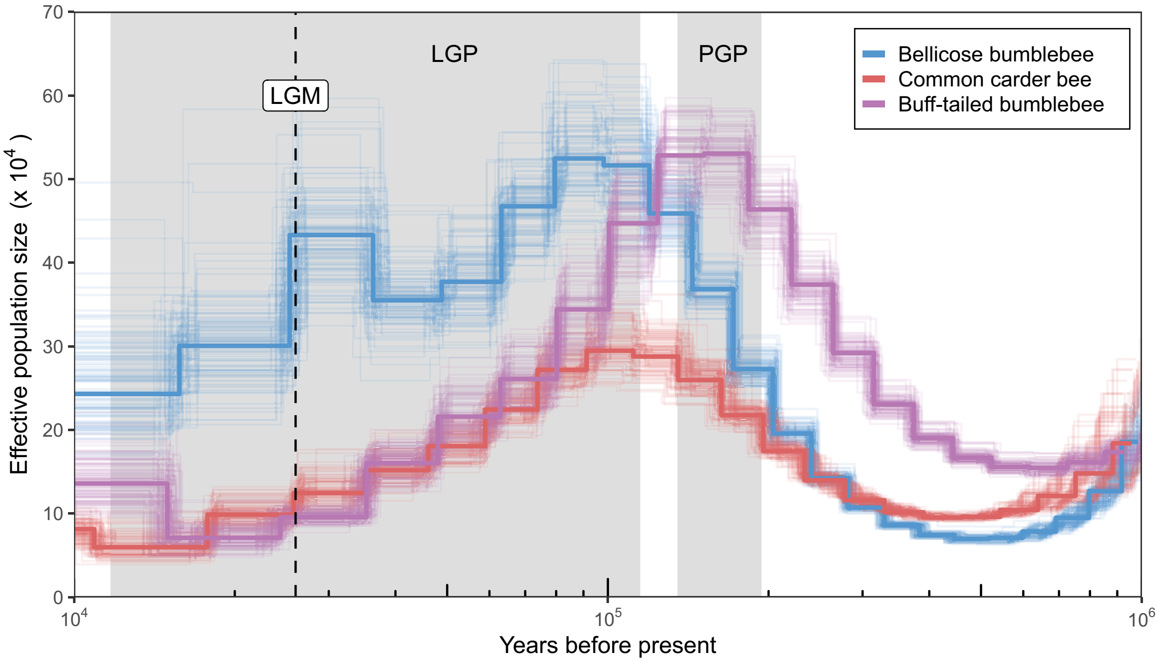
Functional annotation using eggNOG on the 17 pseudochromosomes covered a total of 13 341 transcripts and 11 380 proteins (Data S3), whereas InterProScan covered a total of 15 115 transcripts and 13 176 proteins (Data S4). The higher annotation coverage obtained using InterProScan (82%) in comparison to eggNOG (71%) supports previous comparisons done for lepidopteran genomes (McCartney et al. 2024). From all the functionally annotated genes, InterProScan exclusively annotated 2317 proteins, whereas eggNOG exclusively annotated 521 proteins, with an overlap of 10 859 proteins. A similar pattern emerged when analyzing unplaced contigs (Data S5–S6). This shows that combining independent methods may provide a more comprehensive functional annotation.

Finally, the mitochondrial genome of the bellicose bumblebee spanned 19 060 bp, encompassing all typical 13 mitochondrial protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes, and a large A + T-rich region (Fig. S3). The mitochondrial genome size recovered is within the range reported for other bumblebees, and the arrangement of mitochondrial genes was similar to that of other species within subgenus *Thoracobombus* (Gonçalves et al. 2024).

Conservation genomics of the bellicose bumblebee

We analyzed the novel bellicose bumblebee genome alongside two previously published diploid bumblebee genomes to gain insights into the genetic diversity of this threatened species (Fig. 2). Heterozygosity varied across genomes: the mean heterozygosity of the bellicose bumblebee (2.7 per kbp) was lower than that of the common carder bee (3.9 per kbp) but comparable to the buff-tailed bumblebee (2.9 per kbp), supporting our initial hypotheses. The lower heterozygosity observed in the bellicose bumblebee compared to the common carder bee suggests a smaller N_e (Wright 1931). The common carder bee is widespread across the western Palearctic region, while the bellicose bumblebee is restricted to southern South America. Neutral theory predicts that genetic diversity should correlate with population size (Wright 1931;

Fig. 3. Historical changes in effective population size (N_e) inferred for select bumblebee species. The bellicose bumblebee (*Bombus bellicosus*, in blue) exhibits demographic patterns of population expansion during the Penultimate Glacial Period (PGP) and the Last Glacial Period (LGP), followed by a notable decline post-Last Glacial Maximum (LGM). In contrast, the Palearctic common carder bee (*Bombus pascuorum*, red) and buff-tailed bumblebee (*Bombus terrestris*, purple) show significant population contractions during the LGP, with a subsequent expansion post-LGM in the case of the common carder bee. Lighter lines represent bootstrapped pairwise sequentially Markovian coalescent model runs, while darker lines indicate the mean across all replicates.



Leffler et al. 2012), and intraspecific genetic variation and heterozygosity can be used as proxies for species abundance and geographic range size (Nazareno et al. 2017; Grundler et al. 2019; Qu et al. 2020). In contrast, the buff-tailed bumblebee showed heterozygosity levels comparable to the bellicose bumblebee despite its broad distribution and abundance. This is likely due to artificial selection and genetic drift from its extensive use in greenhouse pollination worldwide (Kardum Hjort et al. 2022). Although the reference genome specimen was collected in the wild (Crowley et al. 2023*b*), historical gene flow between wild and managed buff-tailed bumblebee colonies is well-documented (e.g., Seabra et al. 2019; Franchini et al. 2023). Decades of intensive use for crop pollination in Europe (Chandler et al. 2019) complicates efforts to assess the species' genetic diversity, as we cannot determine how much of its genome originates from managed colonies.

We also scanned the genome for ROH, which are contiguous regions with negligible or completely absent heterozygosity. In conservation genetics, the presence of long ROH (over 1 Mbp) indicates recent inbreeding, while an excess of short ROH may suggest ancient inbreeding events (Howrigan et al. 2011). However, homozygous regions can also arise naturally through selection, even in outbred populations (Curik et al. 2014; Dixit et al. 2020). Most of the ROH in the three bumblebee species ranged from 10 to 300 kbp (Fig. 2). The longest ROH were found in the common carder bee (520 and 450 kbp) and the bellicose bumblebee (600 kbp). Thus, none of the individuals analyzed here showed signs of contemporary or ancient inbreeding based on ROH length, refuting our initial hypothesis. This is promising for the bellicose bumblebee, as it suggests that the population from which this specimen originated maintains considerable genetic diversity. However, it is important to note that extinction risks due to anthropogenic factors—such as habitat fragmentation, pollution, and pesticide use—may reduce population sizes without directly compromising genetic diversity in the short term (Anderson et al. 2010; Struebig et al. 2011). This decoupling highlights the need for conservation measures that address immediate threats to population numbers before genetic erosion occurs.

PSMC analysis revealed distinct demographic histories for the bumblebee species (Fig. 3). The bellicose bumblebee exhibited population size expansion during the early Last Glacial Period (LGP), followed by a sharp contraction after the Last Glacial Maximum (LGM). Previous models for other Brazilian bumblebee species also reported this pattern of expansion during the LGP (Françoso et al. 2016*b*) and decline after the LGM (Krechemer and Marchioro 2020). Specifically, the bellicose bumblebee is estimated to have lost over a third of its range since the LGM (Krechemer and Marchioro 2020), evidencing the sensitivity of this species to warmer temperatures. While bumblebees are generally cold-adapted insects, the effects of climatic changes during the LGP and LGM varied by region. In the Neotropics, colder temperatures favored population expansion for most Brazilian species (Krechemer and Marchioro 2020; Santos Júnior et al. 2022). However, in the Palearctic, the extreme cooling during the LGP exceeded the tolerable limits for many bumblebee populations, leading to range shrinkage and fragmentation (Dellicour et al. 2017). Our PSMC results further support this, showing dramatic declines in the Palearctic common carder bee and buff-tailed bumblebee following the onset of the LGP (Fig. 3). These findings underscore how regional differences in climate severity during glacial periods drove contrasting population dynamics in Palearctic and Neotropical bumblebees.

While our findings highlight the impact of past climatic changes on the population dynamics of the bellicose bumblebee, ongoing and future environmental changes pose additional threats to this species. Global warming projections estimate an increase in global temperatures of up to 4.5 °C by the end of the century under high-emission scenarios (IPCC 2021), which could further reduce the northern range of the bellicose bumblebee and force populations to migrate southward (Martins et al. 2015). Such a shift could also bring the species into closer contact with the invasive buff-tailed bumblebee that has potential to advance in southern South America (Acosta et al. 2016), leading to competition for resources and increased susceptibility to diseases, as observed in other regions (Plischuk et al. 2011; Arbetman et al. 2013; Morales et al. 2013; Schmid-Hempel et al. 2014; Aizen et al. 2019). Additionally, the expected rise in temperatures may exacerbate overheating within the wax-lined nests of bellicose bumblebees, further challenging their reproductive success (Martins et al. 2015; Krechemer and Marchioro 2020), although this scenario requires further investigation. Recent studies on the microbiome of the bellicose bumblebee suggest that this species is particularly sensitive to both pathogens (e.g., *Nosema*), and environmental pollutants (Fernandez De Landa et al. 2024). Furthermore, there remains a critical gap in our understanding of how synergistic stressors—such as pesticides, pathogens, and warming—might together impact the health and survival of wild pollinators (Cameron and Sadd 2020; Dicks et al. 2021; Straub et al. 2022). Understanding these threats is essential to developing effective conservation strategies that can safeguard the long-term persistence of the bellicose bumblebee in a rapidly changing world.

Collectively, our results suggest that the bellicose bumblebee may still retain genetic diversity, even in light of its local extinction in the northern part of its range. The observed levels of heterozygosity and the absence of inbreeding signs are promising. However, the specimen sequenced here was collected from a region that is projected to become unsuitable for this species under future climate scenarios (Martins et al. 2015). This raises concerns about the potential loss of genetic diversity in populations in these vulnerable areas and emphasizes the need of studying southern populations, where the species may persist under climate change. Our analysis was based on a single individual from the center of the species' range (Fig. 1), where populations generally exhibit higher genetic diversity and gene flow. Peripheral populations, in contrast, are often more genetically isolated, as predicted by the center–periphery hypothesis (Eckert et al. 2008; Freitas 2022). This may explain the relatively high heterozygosity, low number of ROH, and large N_e estimated for the bellicose bumblebee in the recent past (Figs. 2 and 3). Nonetheless, peripheral populations are more vulnerable to disturbances (Hampe and Petit 2005). For instance, the local extinction of the bellicose bumblebee in the northern part of its distribution reflects this increased vulnerability. Similar patterns are observed in other bumblebee species, such as the Brazilian *B. pauloensis*, with central populations being more stable and genetically diverse, while peripheral populations face a higher risk of decline (Françoso et al. 2019). The difficulty in locating bellicose bumblebee specimens, even with the support of citizen scientists, highlights the rarity and ongoing decline of this species, particularly in Brazil. Addressing these challenges will require further studies involving multiple individuals from across the species' range to fully assess its genetic health and conservation status. Additionally, adopting non-lethal sampling methods for genetic studies is crucial (Holehouse et al. 2003), as these approaches are feasible and enable broader geographic and demographic sampling without further endangering populations.

Our study represents a crucial first step in utilizing genetic resources for monitoring bellicose bumblebee populations and informing conservation strategies. The signs of genetic diversity identified here underscore the importance of generating further resources to ensure the long-term survival of this species. Implementing protection programs that prioritize population connectivity and habitat preservation is paramount for safeguarding the bellicose bumblebee. By leveraging this genome and the efforts of citizen scientists, we take an important step toward the conservation of a species imperiled by the ongoing and accelerating impacts of human activity.

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Community involvement

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Data availability

The data that support the findings of this study are available as supplementary material at https://github.com/leonardotgoncalves/bellicosus. The reads and the genome assembly generated are available on GenBank under BioProject PRJNA997307.

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Competing interests

The authors declare there are no competing interests.

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