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Short Communication

The genome sequence of the endemic Mexican common mustached Bat, *Pteronotus mexicanus*. Miller, 1902 [Mormoopidae; *Pteronotus*]

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ABSTRACT

We describe here the first characterization of the genome of the bat *Pteronotus mexicanus*, an endemic species of Mexico, as part of the Mexican Bat Genome Project which focuses on the characterization and assembly of the genomes of endemic bats in Mexico. The genome was assembled from a liver tissue sample of an adult male from Jalisco, Mexico provided by the Texas Tech University Museum tissue collection. The assembled genome size was 1.9 Gb. The assembly of the genome was fitted in a framework of 110,533 scaffolds and 1,659,535 contigs. The ecological importance of bats such as *P. mexicanus*, and their diverse ecological roles, underscores the value of having complete genomes in addressing information gaps and facing challenges regarding their function in ecosystems and their conservation.

1. Introduction

Pteronotus mexicanus is a medium-sized insectivorous vespertilionid bat, whose diagnosis includes the presence of hairs on the upper lip, as well as characteristic ornamentations on the lower lip composed of folds and warts (Fig. 1). The dorsal fur is cinnamon-brown, although variations in this feature may occur depending on the locality (Simmons & Conway, 2001; Patton & Gardner, 2007). It is a restricted and endemic species only found in the Pacific slope of Mexico, from the northern state of Sonora to the central coast of Mexico (Pavan & Marroig, 2016). This species lives in various types of habitats, including deciduous tropical forest, deciduous lowland forests and xerophytic shrubland, among others, having been recorded from sea level up to 1,600 m above sea level (Herd, 1983, Wilson & Mittermeir, 2019). The reproductive season coincides with the seasonal rains, beign a seasonal monoestrous species, with births recorded from December to February, and a gestation period of approximately four months (Smith, 1972; Patton & Gardner, 2007).

Originally, *P. mexicanus* was considered a subspecies within the *Pteronotus parnelli* complex (Smith, 1972; Lewis-Oritt et al. 2001), but recent studies in integrative taxonomy allowed its recognition as a separate species (Van Den Bussche et al. 2002, Van Den Bussche & Weyandt, 2003; Dávalos, 2006; Clare et al. 2013; Thoisy et al. 2014; Pavan & Marroig, 2016). Its sister species is *Pteronotus mesoamericanus*, which shares a similar morphology and has an overlapping distribution area, is considered a cryptic species (Clare, 2011). However, the divergence between mtDNA sequences, demonstrated a high geographical

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structure and limited gene flow, allowing their separation into two different species (Pavan & Marroig, 2016). Genome sequencing has been performed for *P. mesoamericanus* collected from Belize (GenBank accession: GCF_021234165.1).

The use of genetic tools to assess genomes/transcriptomes in bats has increased exponentially in recent years, making possible to analyze the whole genome of bat species and significantly contributing to a better understanding of evolutionary processes. Genomic analysis facilitates the identification of coding regions and the discovery and qualification of differential gene expression (Gutiérrez-Guerrero et al., 2020). Consequently, it enhances our understanding of the relationships between different species through phylogenetic and evolutionary studies. Additionally, this aids in identifying viruses and diseases present in animals and their potential transmission to other species. It also supports population conservation efforts based on genetic diversity studies.

Particular applications of comparative genomes can help assess phylogenetic relationships among species (Lei & Dong, 2016), identify correlations between energy metabolism and self-powered flight, or even uncover insights into the bat immune system response (Zhang et al., 2013), among other objectives. Collecting some bat species can be very difficult, which is why the only available source of tissue for sequencing comes from museum specimens. In addition, the use of high-throughput, short-read sequencing of samples from museums and biological collections is important, as these samples are often many years old and the DNA is generally fragmented. This has accelerated the pace of collections-based genomics, with researches opting to sequence genomes with short reads (Roycroft et al., 2022).

The Bat 1 K Genome Project is a global effort to sequence and annotate chromosome-level genome assemblies of all living bat species (Teeling et al., 2018). Due to the important ecological roles of bats in ecosystems, prioritizing bat genomes is indispensable for addressing the numerous challenges related to human well-being, ecosystem function, and biodiversity conservation.

The aim of this study was to provide the first characterization of the genome of *Pteronotus mexicanus,* an endemic species of Mexico, as part of the Mexican Bat Genome Project, an affiliate of the Bat1K Project (Teeling et al., 2018), which specifically focuses on the characterization and assembly of the genomes of endemic bats from Mexico. Both initiatives are dedicated to acquiring comprehensive, well-defined, and assembled genomes of bat species, encompassing those in Mexico and globally.

2. Material and methods

2.1. Sample identification and characteristics

The sequence is derived from the liver tissue of an adult male specimen (catalog number TTU-104773) from the Natural Science Research Laboratory at Texas Tech University. The sample was collected on March 11, 1985, by RJ Baker using a mist net at 8.1 mi N Campo Acosta on HWY 200, in Jalisco, Mexico (georeferenced 19.857371, −105.321281). The collection was allowed under Mexico collection permit number 1547.

The specimen was identified as *P. parnelli* at the time of the field capture, based on gross physical identification, and its mitochondrial cytochrome *b* sequences (NCBI accession KX589903) were later evaluated as *P. mexicanus* based on collection location (Pavan & Marroig 2016).

The animal was euthanized with a pentobarbital overdose. All tissue samples were flash-frozen and maintained in liquid nitrogen until shipment with the cold chain maintained. The gathered specimen is deposited in the mammal collection of Texas Tech University (TTU-104773 = TK 2150567). The harvested tissue was immersed in ethanol and stored at −80 °C until processing.

2.2. Nucleic acid extraction

Genomic DNA extraction was conducted at the Sudmant Lab,

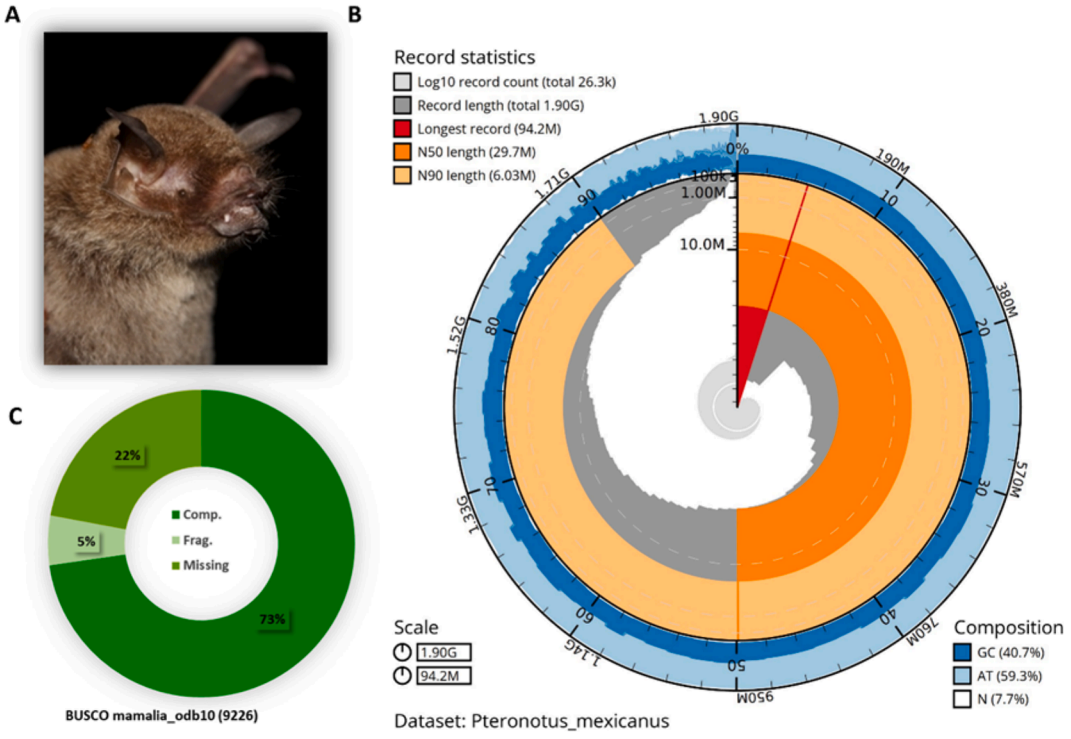

Fig. 1. A) Snailplot representing the metrics and gene completeness of *Pteronotus mexicanus* assembly. The dark grey area represents the distribution of scaffold lengths, the plot radius is scaled to the longest scaffold present in the assembly (65,380,999 bp, shown in red). B) Orange and pale-orange areas represent the N50 and N90 scaffold lengths (50,647,002 and 1,261,894 bp), respectively. The pale grey spiral shows the cumulative scaffold count on a log-scale. The outer area in blue and pale-blue area displays the distribution of GC, AT, and N percentages.

University of California-Berkeley. To eliminate ethanol, a buffer comprising 400 mM NaCl, 20 mM Tris at pH 7.5, and 30 mM EDTA was employed. The process involved gentle agitation for 30 min at room temperature and was repeated twice. After buffer removal, excess liquid was eliminated using a kimwipe. The liver tissue was then transferred to a sterile petri dish, where 1 mL of PBS was added and finely crushed with a sterile knife. The resulting material was moved to a 1.5 mL tube, centrifuged for 10 min at 10,000 g, and the supernatant was discarded. Subsequently, 200 uL of Zymo Research lysis solution and 20 uL of proteinase K were added, followed by overnight incubation at 55 °C with gentle agitation. The mixture was then centrifuged at 10,000 g for 1 min to remove debris and recover the supernatant. The obtained supernatant was placed in a clean tube, and 10 uL of RNAse was added and incubated for 5 min. Magnetic beads and Qiagen W buffer were added in a 1:1 ratio (150 uL), incubated for 1 h, and centrifuged at 10,000 g for 1 min. Tubes were placed on a magnetic stand for 10 min, after which the supernatant was removed without disturbing the tubes. The sample was washed three times with 80 % ethanol, and after the ethanol wash, any remaining ethanol was allowed to evaporate at room temperature. DNA was eluted from the magnetic beads with 100 uL of Qiagen EB solution, and the resulting supernatant was stored at refrigeration temperature.

DNA quantification was performed using a Nanodrop spectrophotometer, and quality assessment was conducted with a Qubit Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Values of 312 ng/microliter of DNA concentration were obtained. Agarose gels were used to corroborate the quality of the extracted samples.

2.3. Sequencing and assembly

Sequencing was conducted with Illumina synthesis technology, on Novaseq 6000 equipment using an S4 flow cell, with a sequence length of 150 bp. PE libraries were constructed following the manufacturer's instructions at Novogene (Sacramento, CA). A total of 641,137,428 PE reads were obtained and quality was assessed using FastQC V.0.11.9 software (Andrews, 2010).

Quality filtering as well as adapter removal, was carried out with the Trimommatic v0.39 program (Bolger et al., 2014). For this purpose, different intervals of the ILLUMINACLIP and SLIDINGWINDOW parameters were tested (relevant parameters used parameters: LEADING:15 TRAILING:15 ILLUMINACLIP:TrueSeq3-PE-2:2:20:10 SLIDINGWINDOW:5:15), to obtain the optimal parameters based on the quality and percentage of reads retrieved. A total of 92.5 % of reads were recovered in *P. mexicanus*.

Assembly was performed with Platanus 2.0 on the Patung server at LANCIS- Instituto de Ecología – UNAM, and scaffolding was achieved through the RagTag 2.1.0 scaffolder using the *Pteronotus mesoamericanus* (GCF_021234165.1 aka *P. parnelli mesoamericanus*) assembly as a reference, and manual assembly curation corrected missing joins and eliminated duplications. The final assembly quality was assessed using QUAST V.5.2.0 Mikheenko et al., 2018) and BUSCO software.

To visually represent the quality metrics and gene completeness, we used BlobToolKit (Challis et al., 2020) to generate a SnailPlot displaying the distribution of the scaffold lengths along with the N50 and N90 values. Additionally, to identify the integrity of the genome assembly and to detect possible contamination, we used BlobToolKit to calculate the GC percent and identify scaffolds with unexpected coverage. To distinguish putative contaminants, we ran blastn v2.12.0 (Camacho et al., 2008) against the NCBI NT database (2023–08-21 version) considering an e-value threshold of 1e-25. Finally, we generated a GC-coverage plot to represent the scaffolds' GC content against their coverage, including the proportion of scaffolds associated with the matched taxa in the NCBI database.

3. Results

The ultimate assembly of *P. mexicanus* (Fig. 1A) encompasses a total length of 1.9 Gb distributed in 110,533 sequence scaffolds. Genome assembly and scaffolds data were named in order of size (Fig. 1B).

The assembly achieved a (Waterhouse et al., 2018) BUSCO v5.3.2 completeness of 72.8 % (single = 72.7 %, duplicated = 0.1 %), using the mamalia_odb10 reference set (Fig. 1C). The N50 scaffold index was 29 Mb (Table 1). Additional summary data for the *P. mexicanus* genome can be found in Table 1.

The taxonomic groups associated with the genome assembly of *P. mexicanus* correspond to the expected genera, as shown in Fig. 2. Most of the taxonomic groups associated with the assemblage correspond to the same genus of *P. mexicanus*, as expected.

4. Discussion

The obtained genome size of *P. mexicanus,* 1.9 Gb, was similar to those reported for other bats (see Gutiérrez-Guerrero et al., 2020). The specimen was identified as *P. parnelli* at time of field capture based on gross physical identification, and its mitochondrial cytochrome *b* sequences (NCBI accession KX589903). The individual was later identified as *P. mexicanus* based on collection location (Pavan & Marroig 2016). The importance and usefulness of genomes is reflected by the fact that it allowed us to verify the identity of the species under study. The methodology we followed allowed us to effectively use an old sample (collected in 1985) from a biological collection. Generating reference genomes from museum samples is crucial, even if the resulting assemblies are incomplete or drafts due to DNA degradation. Despite the challenges in achieving chromosome-complete assemblies, such as those aimed for in the Bat1K project, these efforts are invaluable. They provide a wealth of genetic information that enhances our understanding of historical biodiversity, evolutionary relationships, and genetic variations. Incomplete genomes still offer critical insights that can guide conservation strategies and future research, making them an essential part of genomic science. The followed methodology allowed us to effectively use an old sample (collected in 1985) from a biological collection. These biological collections are a source of historical and biological information of rare or low-abundant species. These specimens are now in some cases the only viable source of genomic data and have accelerated the pace of collections-based genomics (Roycroft et al., 2022). Such specimens are proving to be critical in the fine reconstruction of evolutionary history.

Table 1
Genome data for of *Pteronotus mexicanus.*

Project accession data	
Assembly identifier	JAWWUB000000000
Species	*Pteronotus mexicanus*
Specimen Museum Catalog	TK-150567.1 – TTU 104773
NCBI taxonomy ID	NCBI:txid1884721
BioProject	PRJNA1021227
BioSample ID	SAMN38199800
Isolate information	Male – liver
Raw data accessions	
Hi-C Illumina	SRX23617413
Assembly details	
Assembly accession	JAWWUB010000000
Accession ofAlternative haplotype	N/A
Span (Mb)	1903.1
Number of contigs	1,659,535
Contig N50 length (Mb)	3
Number of scaffolds	110,533
Scaffold N50 length(Mb)	29
Longest scaffold (Mb)	94.2
BUSCO**	C:72.8 %[S:72.7 %,D:0.1 %], F:5.2 %,M:22 %, n:9,226, C≥72 %

** BUSCO scores based on the mamalia_odb10 BUSCO set using v5.3.2. C=complete [S=single copy, D=duplicated], F=fragmented, M=missing, n = number of orthologues in comparison.

Fig. 2. Genome assembly of *Pteronotus mexicanus*: GC-coverage plot. Scaffolds are colored by taxonomic group. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. Staxids are indicated in Supplementary Table 1.

Contributing to cataloging the diversity present in all living bats will allow us to understand the genomic bases of their varied adaptations and their evolutionary history. As part of the Mexican Bat Genome Project, an affiliate of the Bat1K Project (Teeling et al., 2018), providing high-quality, open-access bat genomes that undergo sequencing, assembly, and annotation using standardized laboratory and bioinformatics processes will bring substantial benefits to researchers in different disciplines and could be the basis for numerous future projects.

We believe that providing reference genomes will be beneficial for comparing different groups of species and related species. These efforts will ultimately enhance our understanding and improve conservation efforts for bats.

5. Nucleotide sequence accession number

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAWWUB000000000 and is associated with NCBI BioProject: PRJNA1021227 and BioSample: SAMN38199800 within the GenBank.

6. Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Deuterostomia; Chordata; Craniata; Vertebrata; Gnathostomata; Teleostomi; Euteleostomi; Sarcopterygii; Dipnotetrapodomorpha; Tetrapoda; Amniota; Mammalia; Theria; Eutheria; Boreoeutheria; Laurasiatheria; Chiroptera; Yangochiroptera; Mormoopidae; Pteronotus; *Pteronotus mexicanus,* (NCBI: txid1884721; subordinal taxonomy updated per Teeling (Teeling et al., 2005).

CRediT authorship contribution statement

Guillermo Sánchez-de la Vega: Methodology, Data curation. **Jaime Gasca-Pineda:** Methodology, Data curation. **Anahí Martínez-Cárdenas:** Methodology, Formal analysis, Data curation. **Sonja C. Vernes:** Writing – review & editing, Conceptualization. **Emma C. Teeling:** Writing – review & editing, Conceptualization. **Meike Mai:** Writing – review & editing. **Erika Aguirre-Planter:** Project administration. **Luis E. Eguiarte:** Conceptualization. **Caleb D. Phillips:** Writing – review & editing, Resources. **Jorge Ortega:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2024.148821.

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