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The complete mitochondrial genome and phylogenetic position of the Commissaris's long-tongued bat *Glossophaga commissarisi*

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Well resolved phylogenetic relationships are fundamental to understanding species' evolutionary history, but inferring robust phylogenies can be challenging. The mitochondrial genome has resulted a valuable resource to achieve higher phylogenetic resolution and support. The Commissarisis's long-tongued bat (*Glossophaga commissarisi*) is a widely distributed nectar-feeding bat in the Americas, ranging from Mexico to Colombia and Peru. In this study, we sequenced and assembled the complete mitochondrial genome of the species to characterize its genomic structure, codon usage, and patterns of selection; and to determine its phylogenetic position within Phyllostomidae. We confirmed its phylogenetic position within the genus *Glossophaga* and the family Phyllostomidae. The mitogenome was a circular molecule with a total length of 16,648 bp, containing 13 protein-coding genes (PCGs), two ribosomal genes, 22 transfer RNA genes, and one D-loop or control region (CR). The overall nucleotide composition was A = 32.18%, T = 29.63%, C = 23.97%, and G = 14.22%, with A + T content = 61.81% and G + C content of 38.19%. The phylogenetic tree reconstructed using the 13 PCGs included 61 taxa and recovered *G. commissarisi* as a sister species of *G. leachii* and a fully-supported clade (bv = 100) containing the genus *Glossophaga*. Our study provides a crucial genomic resource for the study of these bats and demonstrates the utility of complete mitogenomes in achieving well-resolved phylogenies for rapidly diversifying mammalian groups.

Keywords: Chiroptera, genomic resources, mitochondrial evolution, molecular phylogenetics, New World bats, selective constraints

Las relaciones filogenéticas bien resueltas son fundamentales para comprender la historia evolutiva de las especies; sin embargo, es un reto obtener filogenias concluyentes. El genoma mitocondrial ha resultado ser un recurso valioso para obtener mayor resolución en las relaciones filogenéticas. El murciélago lengüetón de Commissarisi (*Glossophaga commissarisi*) es un murciélago nectarívoro ampliamente distribuido en las Américas, con un rango de distribución que va desde México hasta Colombia y Perú. En este estudio, secuenciamos y ensamblamos el genoma mitocondrial completo (mitogenoma) de la especie para caracterizar su estructura genómica, uso de codones y patrones de selección; y para determinar su posición filogenética dentro de la familia Phyllostomidae. Confirmamos su posición filogenética dentro del género *Glossophaga* y la familia Phyllostomidae. El mitogenoma fue una molécula circular con una longitud total de 16,648 pb, que contenía 13 genes codificantes de proteínas (PCG), dos genes ribosomales, 22 genes de ARN de transferencia y una región D-loop o región control (CR). La composición nucleotídica general fue A = 32.18%, T = 29.63%, C = 23.97% y G = 14.22%, con un contenido A + T = 61.81% y un contenido G + C de 38.19%. El árbol filogenético reconstruido utilizando los 13 PCG incluyó 61 taxones y recuperó a *G. commissarisi* como una especie hermana de *G. leachii* y un clado con soporte completo (bv = 100) que contenía al género *Glossophaga*. Nuestro estudio proporciona un recurso genómico crucial para el estudio de estos murciélagos y demuestra la utilidad de los mitogenomas completos para obtener filogenias bien resueltas en grupos de mamíferos que se diversifican rápidamente.

Palabras clave: Chiroptera, evolución mitocondrial, filogenética molecular, murciélagos del Nuevo Mundo, recursos genómicos, restricciones selectivas

Phylogenetic relationships are fundamental to understanding evolutionary history, biogeography, and the evolution of ecological traits. However, inferring robust phylogenies can be challenging, often yielding incongruent results between studies that use different datasets (e.g., morphological vs. molecular data) or limited molecular markers (Dávalos *et al.* 2012; Dumont *et al.* 2012). Such phylogenetic incongruence can stem from factors like convergent evolution, incomplete lineage sorting, or the limited phylogenetic signal of individual genes (Rokas and Carroll 2005; Degnan and Rosenberg 2009). The integration of genomic-scale data has emerged as a powerful approach to resolve these conflicts, providing a more comprehensive and statistically robust view of evolutionary relationships by aggregating signals from thousands of independent loci (McCormack *et al.* 2013; Reddy *et al.* 2017).

Among genomic resources, the complete mitochondrial genome (mitogenome) offers a particularly valuable tool for phylogenetic inference at intermediate taxonomic levels (Gissi *et al.* 2008). Mitogenomes provide a set of 37 linked genes that evolve at different rates, combining fast-evolving regions useful for recent divergences with highly

conserved protein-coding genes informative for deeper nodes (Boore 1999). This, coupled with features like rare gene rearrangements, codon usage bias, and patterns of evolutionary selection, makes mitogenomic data superior to single mitochondrial genes for achieving higher phylogenetic resolution and support, especially within rapidly diversifying clades (Cameron 2014; Tan *et al.* 2015).

The New World leaf-nosed bats (family Phyllostomidae) represent a classic example of an adaptive radiation, exhibiting extraordinary ecological diversity – from insectivory and carnivory to frugivory, nectarivory, and even sanguivory – within a relatively recent evolutionary timeframe (Dumont *et al.* 2012; Rojas *et al.* 2016). This rapid diversification has sometimes resulted in unresolved or conflicting phylogenetic hypotheses, particularly within subfamilies (Baker *et al.* 2012; Dávalos *et al.* 2014). The subfamily Glossophaginae (nectar-feeding bats) is a key component of this radiation. Within it, the genus *Glossophaga* is widespread and species-rich, yet the phylogenetic relationships among its species remain partially unresolved due to limited genomic data (Hoffmann *et al.* 2019). The Commissaris's long-tongued bat, *Glossophaga commissarisi* (Gardner 1962), is a widely distributed nectar-feeding bat found from Mexico to Peru, also acting as a facultative insectivore (Sánchez-Casas and Alvarez 2000). Despite its abundance, no complete mitogenome was available for this species, creating a gap in resources needed for phylogenetic analyses of the genus.

To address this gap, we sequenced, assembled, and annotated the first complete mitochondrial genome of *G. commissarisi* with the following objectives: (1) to characterize its genomic structure, codon usage, and patterns of selection; and (2) to determine its phylogenetic position within Phyllostomidae using a phylomitogenomic approach. We predicted that the mitogenome of *G. commissarisi* would exhibit structural conservation and strong purifying selection typical of functional mitochondrial genomes. Furthermore, we predicted that the use of complete mitogenomic data would provide high statistical support for resolving its position as sister to *G. leachii* and for clarifying relationships within Glossophaginae, offering a superior resource compared to single-gene studies.

Materials and methods

Mitogenome sequencing and assembly. Muscle tissue from an adult male of *G. commissarisi* (Figure 1) was collected in the Ejido Loma Bonita, shore of the Lacantun River, municipality Ocosingo, Chiapas, Mexico (Latitude 16.1014583° N, Longitude -91.00196° W), in March 2017 and preserved as a standard museum voucher specimen (skin and skeleton) following established practices (Sikes *et al.* 2011). The tissue was stored at the Mammal tissue collection of El Colegio de la Frontera Sur (ECO-SC-M 8490). Genomic DNA was extracted using a modified phenol-chloroform protocol (Sambrook and Russell 2001). DNA quality was assessed with a Nanodrop 2000 and a quantified Qubit 4 fluorometer. A pair-end (PE, 150 bp) shotgun library was constructed and sequenced on an Illumina NovaSeq 6000 platform at Novogene (Sacramento, CA). 68,005,078 reads in FASTQ format were generated and utilized for *de novo* assembly of the mitochondrial genome (Illumina 2020).

To characterize the genomic structure and codon usage of the mitochondrial genome of *G. commissarisi* we obtained a *de novo* assembly with GetOrganelle v1.7.7.0 with k-mer sizes of 21, 45, 65, 85, and 105, using the mitochondrial genome (seed file) of congeneric *Glossophaga mutica* (Genebank: OR263465.1) (Jin *et al.* 2020). To guarantee a high-quality genome, we analyzed the depth of coverage. The annotation of the complete mitochondrial genome was performed using the web server MITOS2 hosted in Galaxy with the vertebrate code (usegalaxy.org; Donath *et al.* 2019). Nucleotide composition and skew curves of the complete mitogenome and the control region were estimated using a custom R script (R Core Team 2022). We visualized the circular genome using Proksee (Grant *et al.* 2023). Using the Codon Usage web server (vertebrate mitochondrial code), we analyzed PCG codon usage, then calculated amino acid frequencies and RSCU with Ezcodon on the Ezmito web server (http://ezmito.unisi.it/ezcodon; Cucini *et al.* 2021). Mitochondrial tRNA secondary structures, predicted by MiTFi, were visualized using FORNA (Jühling *et al.* 2012; Kerpedjiev *et al.* 2015).

To characterize the patterns of selection in each mitochondrial PCG, we estimated the nonsynonymous (Ka) and synonymous (Ks) substitution rates for each mitochondrial PCG using KaKs_Calculator v2.0.1 to assess selective constraints. The resulting Ka/Ks ratio (ω) for each gene, calculated from pairwise comparisons with *G. leachii* and *G. mutica*, indicated neutral evolution ($\omega = 1$), purifying selection ($\omega < 1$), or positive selection ($\omega > 1$). Calculations employed the γ-MYN model to account for variable mutation rates across sequences (Wang *et al.* 2020).

The control region was examined for microsatellites using the Microsatellite Repeats Finder (Bikandi *et al.* 2004) and for tandem repeats using Tandem Repeats Finder (Benson 1999). The secondary structure of this region was subsequently predicted with FORNA (Kerpedjiev *et al.* 2015).

Phylomitogenomics of Glossophaga commissarisi and family Phyllostomidae. To determine the phylogenetic position of *G. commissarisi* within Phyllostomidae, we used a dataset comprising 55 complete mitochondrial genomes. The ingroup consisted of 51 mitogenomes representing 46 species from the family Phyllostomidae, including our newly sequenced *G. commissarisi*. For the outgroup, we selected four representative species from families closely related to Phyllostomidae, based on recent molecular phylogenies: *Mystacina tuberculata* Mystacinidae), *Myotis nigricans* (Vespertilionidae), *Noctilio leporinus* (Noctilionidae), and *Pteronotus rubiginosus* (Mormoopidae) (Shi and Rabosky 2015).

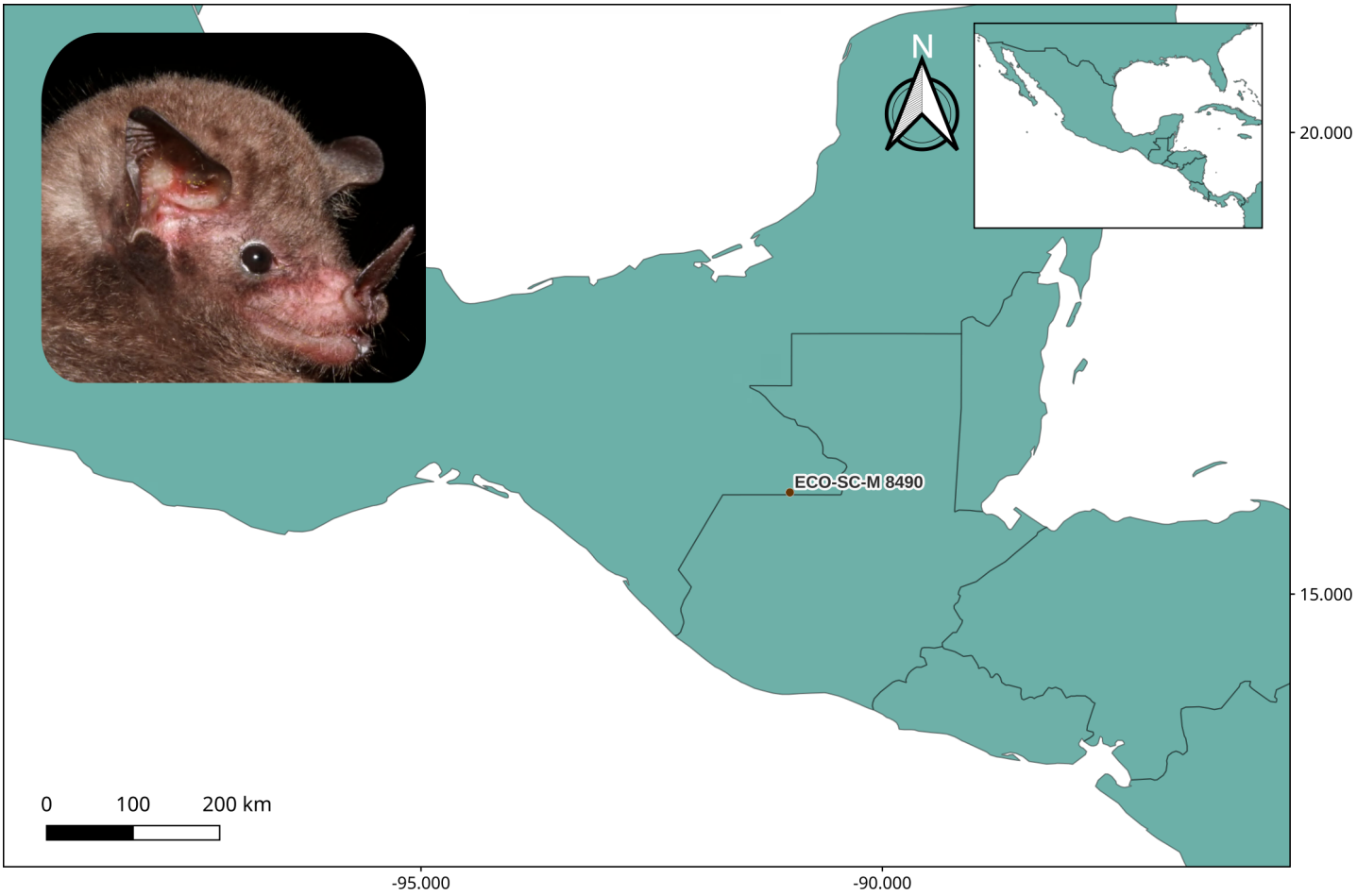

Figure 1. Image of *Glossophaga commissarisi* and its sampling location within the distribution of the species. Collection voucher is denoted in the coordinate label (ECO-SC-M 8490). Photo by Juan Cruzado, used with permission.

A maximum-likelihood (ML) phylogeny was reconstructed using the MitoPhAST v3.0 pipeline (Tan *et al.* 2015). This tool automatically extracted and generated a concatenated and partitioned amino acid alignment from the 13 protein-coding genes (PCGs) across all taxa. This final alignment was then used to build the ML tree with IQ-TREE (Nguyen *et al.* 2015), which automatically selected the best-fit model of protein evolution. The topological robustness of the inferred tree was evaluated with 1,000 bootstrap replicates (Felsenstein 1985).

Results

The mitochondrial genome of Glossophaga commissarisi

The mitogenome (Genbank: PX387959) of Commissaris's long-tongued bat *Glossophaga commissarisi* is 16,648 bp in length (average coverage of 1,684 x) and encodes 37 genes, 13 PCGs, 22 tRNA genes, and two rRNA (rrnS and rrnL) genes, and a 1,202 bp long non-coding region (Table 1; Figure 2; Supplementary Figure 1). Most of the PCGs and tRNAs are encoded on the heavy (H) strand, while the NAD6 gene and eight tRNAs (trnaQ, trnaA, trnaN, trnaC, trnaY, trnaS2, trnaE, and trnaP) are encoded in the light strand (L) (Table 1).

The Control Region in *Glossophaga commissarisi* spans 1,202 bp in length with an A + T content of 59.65%. Microsatellites Repeat Finder analysis identified eight repeats in the CR including five di-nucleotide motifs (AT, TA, CT, CC) and one tri-nucleotide motif (CCA) (Supplementary Table 4). Likewise, Tandem Repeat analysis found a tandem repeat region in the CR spanning positions 776-898 in approximately 10 copies. The motif sequence is CGTATACGCCTA, with base composition A = 24, C = 33, G = 17, T = 25 (Supplementary Table 5).

All tRNA genes exhibit anticodon, acceptors, DHU, and TΨC stems (Supplementary Figure 5) and display a cloverleaf secondary structure, except for tRNA-Serine 1, which usually lacks the DHU arm.

The studied rrnS (12s) and rrnL (16s) genes in *G. commissarisi*'s mitochondrial genome are 971 bp long and 1,573 bp long respectively (see Table 1 of main text). The rrnS gene is positioned between trnaF and trnaV genes, while rrnL is located between trnaV and trnaL2 genes. These genes exhibit an AT composition of 59.94% (12S) and 60.20% (16S).

The nucleotide composition of the positive DNA strand of the mitochondrial genome was as follows: A = 32.18%, T = 29.63%, C = 23.97%, and G = 14.22%, resulting in an overall A + T content of 61.81% and G + C content of 38.19%. The AT skew we observed in the mitogenome is 0.041 (Supplementary Table 1).

The amino acids in each of *Glossophaga commissarisi*'s PCGs are encoded by at least two different codons

Table 1. Characteristics of the mitochondrial genome of *Glossophaga commissarisi*. Continuity refers to the number of overlapping nucleotides between consecutive features: positive values indicate gene overlap, negative values indicate intergenic gaps, and zero indicates adjacent features. Stop codons that are shown with parentheses denote incomplete stop codons.

Feature	Type	Start	End	Strand	Length (bp)	Start codon	Stop codon	Anticodon	Continuity
Phe	tRNA	1	68	+	68			GAA	0
12S rRNA	rRNA	69	1039	+	971				0
Val	tRNA	1040	1107	+	68			TAC	0
16S rRNA	rRNA	1108	2680	+	1573				0
Leu	tRNA	2682	2756	+	75			TAA	1
NAD1	PCG	2759	3715	+	957	ATG	TAA		2
Ile	tRNA	3715	3783	+	69			GAT	-1
Gln	tRNA	3781	3853	-	73			TTG	-3
Met	tRNA	3853	3921	+	69			CAT	-1
NAD2	PCG	3922	4965	+	1044	ATT	TAG		0
Trp	tRNA	4966	5033	+	68			TCA	0
Ala	tRNA	5038	5106	-	69			TGC	4
Asn	tRNA	5108	5180	-	73			GTT	1
OL		5183	5213	-	31				2
Cys	tRNA	5213	5278	-	66			GCA	-1
Tyr	tRNA	5279	5344	-	66			GTA	0
COX1	PCG	5346	6890	+	1545	ATG	TAA		1
Ser	tRNA	6888	6958	-	71			TGA	-3
Asp	tRNA	6962	7028	+	67			GTC	3
COX2	PCG	7029	7712	+	684	ATG	TAA		0
Lys	tRNA	7716	7784	+	69			TTT	3
ATP8	PCG	7786	7989	+	204	ATG	TAA		1
ATP6	PCG	7947	8627	+	681	ATG	TAA		-43
COX3	PCG	8627	9410	+	784	ATG	T(AA)		-1
Gly	tRNA	9411	9480	+	70			TCC	0
NAD3	PCG	9481	9828	+	348	ATT	TAA		0
Arg	tRNA	9829	9896	+	68			TCG	0
NAD4L	PCG	9897	10193	+	297	ATG	TAA		0
NAD4	PCG	10187	11564	+	1378	ATG	T(AA)		-7
His	tRNA	11565	11632	+	68			GTG	0
Ser	tRNA	11633	11691	+	59			GCT	0
Leu	tRNA	11693	11762	+	70			TAG	1
NAD5	PCG	11763	13592	+	1830	ATA	TAA		0
NAD6	PCG	13567	14094	-	528	ATG	TAA		-26
Glu	tRNA	14096	14164	-	69			TTC	1
CYTB	PCG	14174	15313	+	1140	ATG	AGA		9
Thr	tRNA	15314	15380	+	67			TGT	0
Pro	tRNA	15380	15446	-	67			TGG	-1
OH		15746	16019	+	274				299
CR	D-loop	15447	16648		1202				

(Supplementary Table 2), with a preference for codons ending in adenine or thymine, while codons ending in guanine are less frequently used. Relative synonymous codon usage (RSCU) and amino acid frequency are summarized (Supplementary Figure 2). The most frequently used codons include ATA (Ile), TTT (Phe), and AAA (Lys), whereas CGG (Arg) and GCG (Ala) are among the least frequently used codons.

Analysis of Ka/Ks ratios for all mitochondrial PCGs (Supplementary Figure 3; Supplementary Table 3) revealed values below 1 ($P < 0.001$). CYTB, COX1, COX2, and NAD4L exhibited the lowest Ka/Ks ratios, whereas ATP8, NAD1, and NAD6 showed relatively higher values. The average Ka/Ks across all 13 PCGs ranged from 0.016 to 0.529, depending on the gene and comparison.

Phylomitogenomics of Glossophaga commissarisi. The ML phylogenetic analysis recovered the expected relationships within Phyllostomidae with high bootstrap support (Figure 3). Within the nectarivorous Glossophaginae, *Glossophaga* sequences clustered

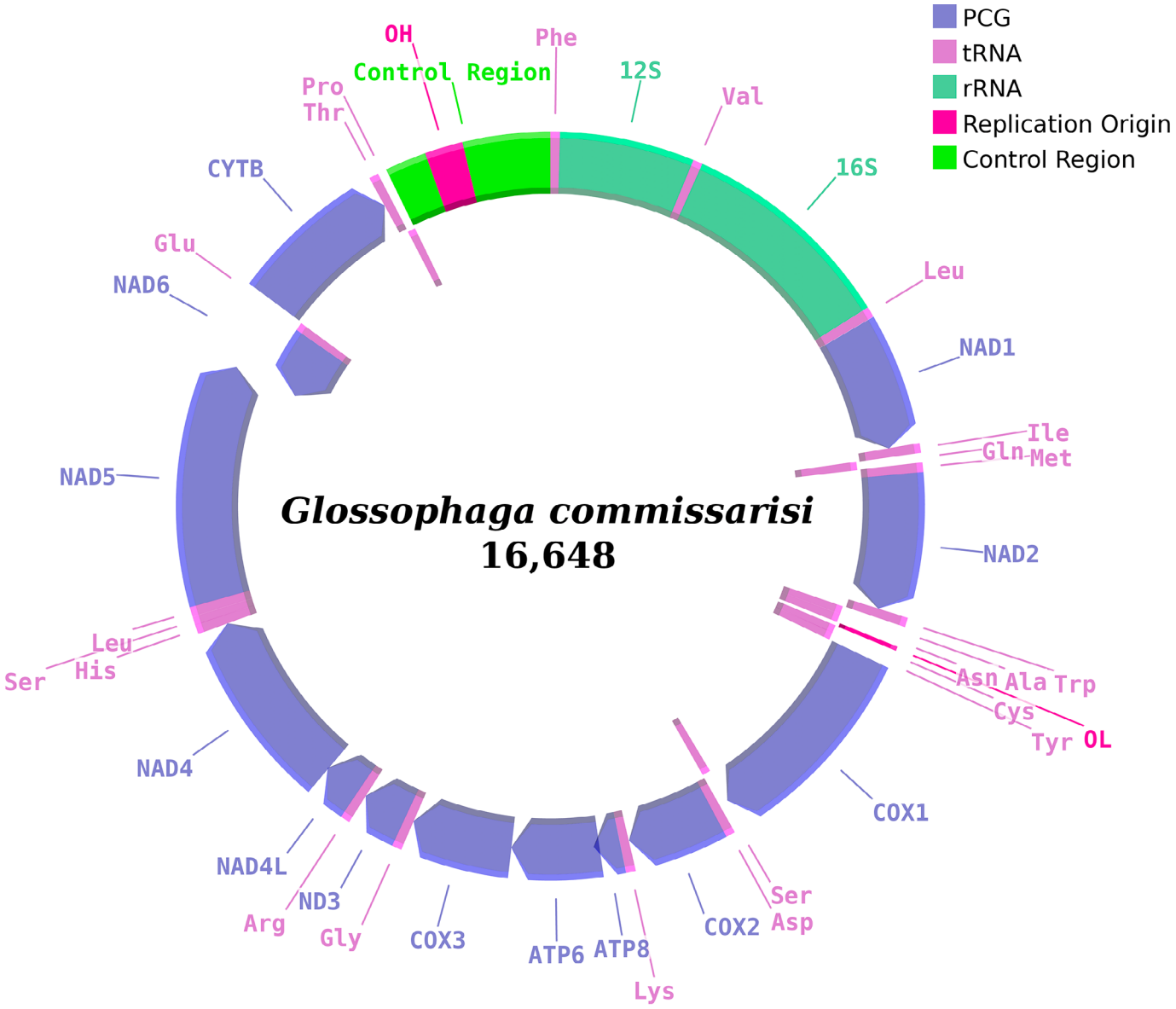

Figure 2. Circular representation of the mitochondrial genome of *Glossophaga commissarisi*. Colors indicate the composition and arrangement of genes. The origins of replication of the light strand (OL) and heavy strand (OH) are annotated in the figure.

together (bv = 100), with *G. soricina* sister to the *G. mutica* clade, and *G. leachii* and *G. commissarisi* forming a strongly supported subclade (bv = 99.3). *Leptonycteris* species were sister to Glossophaginae (bv = 99.9). Other subfamilies, including Phyllostominae, Stenodermatinae, and Lonchorhininae, were recovered with variable support among nodes (bv = 99.6–100).

Discussion

The mitochondrial genome of Glossophaga commissarisi. The gene arrangement in the *Glossophaga commissarisi* mitochondrial genome is similar to those previously reported for phyllostomids and species of the subfamily Glossophaginae (Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025). The Control Region is moderately shorter than congeneric species in *Glossophaga* (Supplementary Figure 4) (Rocamontes-Morales *et al.* 2025). The length and structural features of the tRNA genes resemble those observed in congeneric *Glossophaga*, other phyllostomids and beyond (Meganathan *et al.* 2012; Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025).

The overall nucleotide composition is consistent with the range observed in congeneric *Glossophaga* species, and other Phyllostomids (Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025). Likewise, the observed codon usage patterns have been observed in other congeneric species and other Phyllostomid bats (Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025).

Analysis of evolutionary pressures shows that the mitogenome of *Glossophaga commissarissi* has evolved

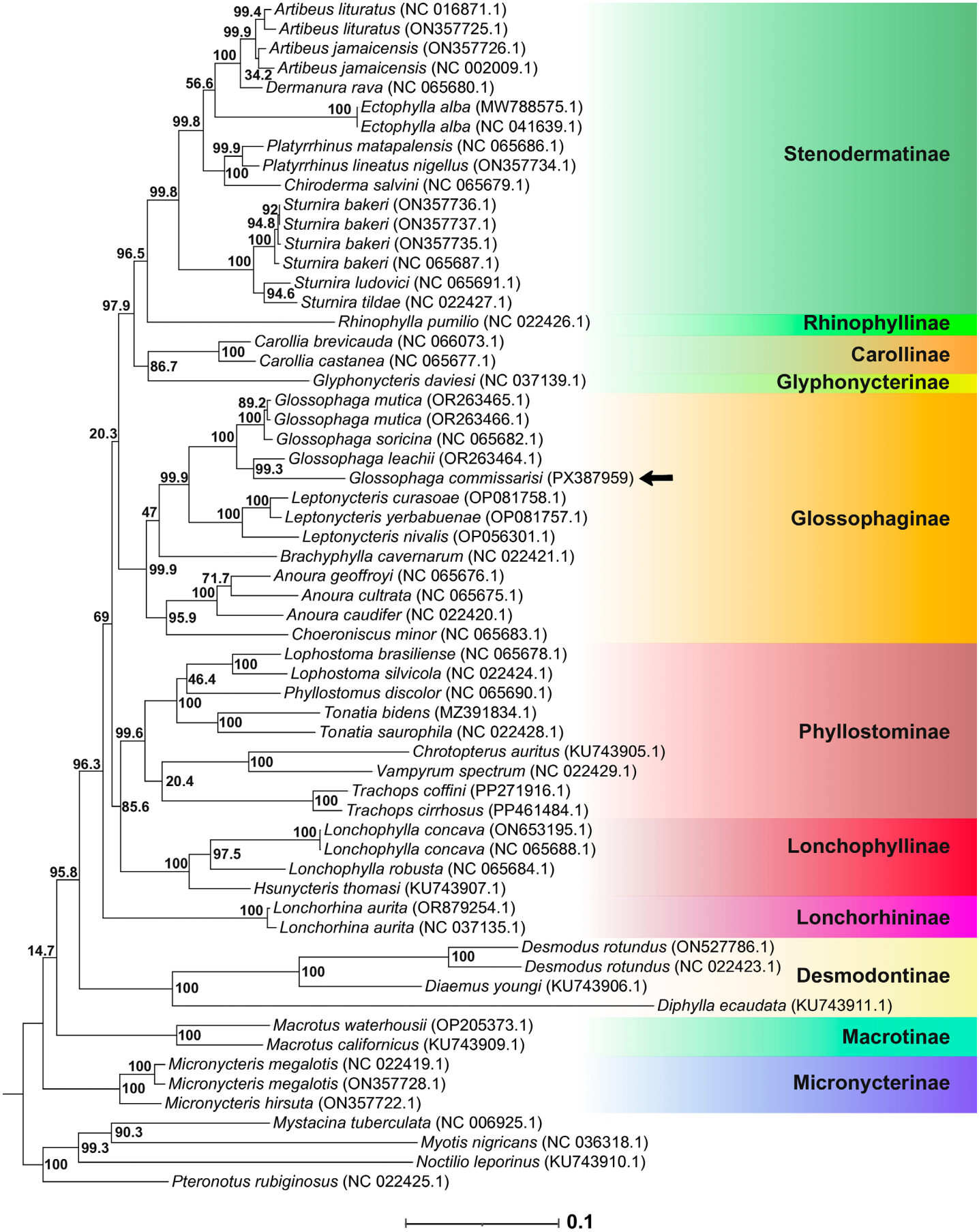

Figure 3. Maximum-likelihood phylogeny of the Phyllostomidae based on mitochondrial genomes. The newly assembled *Glossophaga commissarisi* genome from this study is highlighted with an arrow. Accession numbers are provided in parentheses for all species. Node support is indicated by bootstrap values (shown above/below branches). Species from the Mystacinidae, Vespertilionidae, Noctilionidae, and Mormoopidae families were used as an outgroup.

under strong purifying selection, as evidenced by Ka/Ks ratios below 1 for all 13 protein-coding genes. This finding is consistent with patterns documented across other phyllostomid bats, supporting the conserved functional role of these mitochondrial genes (Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Camacho et al. 2022; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025).

Phylomitogenomics of Glossophaga commissarisi. Our phylogenetic results are largely congruent with previous studies based on concatenated mitochondrial genes (e. g., CYTB, ND2) and multi-locus nuclear datasets, which consistently recover *Glossophaga* as a monophyletic group and place *G. commissarisi* as sister to *G. leachii* (Hoffmann *et al.* 2019; Rocamontes-Morales *et al.* 2025). However, the use of complete mitogenomes in this study provided substantially higher nodal support across the phylogeny, particularly for deeper relationships within Phyllostomidae. For example, the clade containing *G. commissarisi* and *G. leachii* received a bootstrap value of 99.3, and monophyly of Glossophaginae was recovered with full support (bv=100). This contrasts with some earlier studies using fewer mitochondrial markers, where support for these same models was moderate or variable (e.g., Hoffmann *et al.* 2019). The increased signal provided by the concatenated alignment of all 13 protein-coding genes likely contributed to this improved resolution, underscoring the utility of phylomitogenomic approaches in resolving relationships within rapidly diversifying groups like the phyllostomid bats.

Conclusions

In this study, we assembled and annotated the complete mitochondrial genome of *G. commissarisi*. A phylogenetic tree was reconstructed based on all translated PCGs and supports *G. commissarisi* as sister to *G. leachii,* also forming a well-supported clade with *Glossophaga* and subfamily Glossophaginae. These findings provide genetic resources for future studies on the complex and seldom-studied *Glossophaga* genus. We recommend that future research sequence remaining congeners to resolve the comprehensive phylogenetic relationships within the entire genus.

Ethical approval

The material used in this study does not involve ethical conflicts. Tissue samples were donated by the Mammal Tissue Collection of El Colegio de la Frontera Sur, Unidad San Cristóbal de Las Casas, managed by Consuelo Lorenzo. Tissue was collected with collection permit SGPA/DGVS/01186/17 of Consuelo Lorenzo. *G. commissarisi* is a non-endangered species, and all procedures followed the ethical standards outlined in the ARRIVE guidelines and the American Society of Mammalogists (Sikes *et al.* 2011). The protocol for this research project was reviewed and approved by the ECOSUR Ethics Committee for Research (CEI-05-07-2023b).

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Data availability statement

The mitochondrial genome sequence can be accessed via accession number PX387959 in the GenBank of NCBI. The associated BioProject and SRA numbers are PRJNA1021227 and SAMN51504582. The tissue for *G. commissarisi* (ECO-SC-M 8490) was stored at the ECOSUR, Unidad San Cristobal de Las Casas mammal tissue collection managed by Consuelo Lorenzo (clorenzo@ecosur.mx).

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