Gene 850 (2023) 146925

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Complete mitochondrial genome of *Phoneutria depilata* (Araneae, Ctenidae): New insights into the phylogeny and evolution of spiders

Carlos F. Prada [a,*], Nicolas A. Hazzi [b], Gustavo Hormiga [b], Felipe Cabarcas [c,d], Lida M. Franco [e]

[a] Grupo de investigación de Biología y ecología de artrópodos, Facultad de Ciencias, Universidad del Tolima, Colombia
[b] The George Washington University, Department of Biological Sciences, Washington, D.C. 20052, USA
[c] Centro Nacional de Secuenciación Genómica (CNSG), Sede de Investigación Universitaria (SIU), Universidad de Antioquia, Medellín, Colombia
[d] Grupo SISTEMIC, Ingeniería Electrónica, Facultad de Ingeniería, Universidad de Antioquia – UdeA, Medellín, Colombia
[e] Grupo NATURATU Facultad de Ciencias Naturales y Matemáticas, Universidad de Ibagué, Ibagué, Colombia

ARTICLE INFO

Edited by Andre van Wijnen

Keywords:
Comparative genomics
Mitochondrial genomes
Gene order analyses
Spider evolution

ABSTRACT

Spiders (Araneae) are the most abundant terrestrial predators and megadiverse on earth. In recent years, the mitochondrial genome of a great diversity of species has been sequenced, mainly for ecological and commercial purposes. These studies have uncovered the existence of a variety of mitochondrial genome rearrangements. However, there is poor genetic information in several taxonomic families of spiders. We have sequenced the complete genome of *Phoneutria depilata* (Ctenidae) and, based on this, extract the mitogenomes of other ctenid species from published transcriptomes to perform a comparative study among spider species to determine the relationship between the level of mitochondrial rearrangements and its possible relationship with molecular variability in spiders. Complete mitochondrial genomes of eighteen spiders (including eight Ctenidae species) were obtained by two different methodologies (sequencing and transcriptome extraction). Fifty-eight spider mitochondrial genomes were downloaded from the NCBI database for gene order analysis. After verifying the annotation of each mitochondrial gene, a phylogenetic and a gene order analysis from 76 spider mitochondrial genomes were carried out. Our results show a high rate of annotation error in the published spider mitochondrial genomes, which could lead to errors in phylogenetic inference. Moreover, to provide new mitochondrial genomes in spiders by two different methodologies to obtain them, our analysis identifies six different mitochondrial architectures among all spiders. Translocation or tandem duplication random loss (TDRL) events in tRNA genes were identified to explain the evolution of the spider mitochondrial genome. In addition, our findings provide new insights into spider mitochondrial evolution.

1. Introduction

Spiders (Araneae) are members of the ancient class Arachnida, predatory arthropods that have inhabited Earth for approximately 400 million years and are among the most species rich animal groups (with more than 50,000 species described so far) due to their broad diversity, worldwide distribution and conspicuous synapomorphies (Wheeler et al., 2017b; Catalog, 2022; Lüddecke et al., 2022). The order Araneae, with 132 families, is classified into two infraorders, Mesothelae ("primitive spiders") and Opisthothelae (modern spiders). The infraorder Opisthothelae is classified into two suborders Mygalomorphae and Araneomorphae. The family Ctenidae belongs to the suborder Araneomorphae, with 48 genera and 527 species (Kumar et al., 2020; Catalog, 2021). Ctenids are essentially wandering, nocturnal spiders and represent one of top generalist predators of invertebrates and small vertebrates on tropical forest (Hazzi, 2014; Hazzi and Hormiga, 2021). The highly venomous species of the genus *Phoneutria* are some of thebest-known representatives of this family, commonly referred to as "banana spiders," because they often inhabit this crop. Due to their defensive behavior, synanthropic habits, and potent venom, *Phoneutria* species are considered among the most medically important spiders in the world (Foelix, 2010). *Phoneutria* currently comprises nine large (17–48 mm) nocturnal species that are widely distributed in Central America and South America, having its highest species richness in Brazil

Abbreviations: TDRL, Translocation or tandem duplication random loss events; NCBI, National Center for Biotechnology Information; mt, mitochondria; rRNA, ribosomal RNA; tRNA, transfer RNA; PCGs, protein-coding genes; ID, accession identifier; ML, maximum likelihood.

* Corresponding author.
E-mail address: cfpradaq@ut.edu.co (C.F. Prada).

https://doi.org/10.1016/j.gene.2022.146925
Received 27 July 2022; Received in revised form 9 September 2022; Accepted 25 September 2022
Available online 1 October 2022

(Simó and Brescovit, 2001; Martins and Bertani, 2007) and include medically important species such as *P. nigriventer*, responsible for about four thousand bites per year in Brazil (Bucaretchi et al., 2016). Despite their medical and ecological importance, the phylogenetic relationships of Ctenidae remain poorly understood. *Phoneutria depilata* is a widespread species distributed from Central America to the Trans-Andean region of Colombia and Ecuador. This species is mainly found in disturbed habitats associated with both dry and humid tropical forests (0–1700 m), usually on the ground with sparse litter and low vegetation (Hazzi, 2014; Hazzi and Hormiga, 2021). There are human bite records of this species reported in Costa Rica and in banana plantations in Colombia (Florez et al., 2003). All the cases reported have occurred with adults, and most of them have presented mild to moderate envenomation symptoms, with only one patient presenting severe symptoms such as renal failure (Trejos et al., 1971; Florez et al., 2003). *Phoneutria depilata* has been deeply studied in works regarding its geographic distribution(Valerio, 1983; Hazzi et al., 2013), bite accidents to humans (Trejos et al., 1971; Florez et al., 2003), venom toxicity and composition (Estrada-Gomez et al., 2015; Valenzuela-Rojas et al., 2019), natural history(Hazzi, 2014; Valenzuela-Rojas et al., 2020; Sierra Ramírez et al., 2021), and introductions to Europe through banana shipments(Cathrine and Longhorn, 2017; Rozwałka et al., 2017). The animal mitochondrial genome (mitogenome) has been widely used as a source of data for phylogenetic inference at several taxonomic levels due to its rich signal from sequence information and gene arrangement, from populations to phyla, and has been widely used for the resolution of taxonomic controversies (Gissi et al., 2008; Cameron, 2014; Li et al., 2020). The arthropod mitogenome is a compact circular molecule of usually 14–19 kb in size, with 37 genes (13 protein-coding genes, 22 tRNA genes, two rRNA genes), and a noncoding D-loop region. The arrangement of the genes within the arthropod mitogenome is also highly conserved; however, there are some invertebrate lineages with radically rearranged mitochondrial gene order, as in insects (Boore, 1999; Cameron, 2014).

Despite the rapidly increasing mitogenome sequences from diverse invertebrate species, spider mitogenome are poorly studied in all arthropods. To date, 58 mitogenomes of 23 spider families have been accessible in the GenBank database (https://www.ncbi.nlm.nih.gov/), although genome rearrangements are rare in the majority of sider species (Masta and Boore, 2008; Fang et al., 2016; Wang et al., 2016; Zhu and Zhang, 2017; Kumar et al., 2020; Yong et al., 2021). Due to the small number of spiders mitogenomes sequenced, it is not clear both the phylogenetic relationships and the types of rearrangements in this genome. Previous studies have focused on studying phylogenetic relationships from complete or partial mitogenomes in spiders and arachnids; resolving key positions of some orders in the Arachnida and new insights into their ancient evolution (Adrián-Serrano et al., 2021; Ban et al., 2022) or through Genome-scale data sets of hundreds of Araneae species (Kulkarni et al., 2021); providing robust phylogenetic relationships, which allow to elucidate the evolution of this important taxonomic group. However, in these studies there is a low number of species analyzed in some taxa, as in the case of the family Ctenidae, among others. In addition, there is the possibility of having annotation errors in several spiders mitogenomes available in the database that could significantly affect the phylogenetic relationships.

In the present study, we sequenced the complete mitogenome of *Phoneutria depilata* using next-generation sequencing and assembled other spider mitogenomes from transcriptome data available in NCBI public database. Nine mitogenomes were obtained in the family Ctenidae and nine other spider mitogenomes of different taxonomic families. Therefore, this study aims to compare these eighteen *de novo* with 58 spider mitogenomes previously reported to observe their phylogenetic relationships, gene features, gene arrangements, and D-loop region variation that occurred during spider evolution.

2. Materials and Methods

2.1. Sample collection and DNA extraction.

A female adult specimen of *Phoneutria depilata* (initially classified as *P. boliviensis* and reclassified by Hazzi and Hormiga 2021) was collected in September 2019 from Ibague (4°32′22.3″N, 75°05′37.1″W), Tolima, Colombia. The morphological identification of this specimen was performed by previous taxonomic studies (Simó and Brescovit, 2001; Martins and Bertani, 2007; Hazzi and Hormiga, 2021). The specimen was stored in 96% ethyl alcohol at −80 °C in the Molecular Biology Laboratory of the University of Ibague (Ibague, Colombia). The sample was homogenized, and DNA extraction of tissues was performed using the Qiagen DNEasy Tissue kit (Qiagen, Valencia, CA) under the manufacturer's conditions. Genomic DNA concentration was quantified by a dsDNA high-sensitivity kit (Thermo Fisher Scientific, MA, USA) using a Qubit fluorometer.

2.2. Mitogenome sequencing and assembly of Phoneutria depilata

The AIM (Advanced Identification Methods GmbH) (http://www.aimethods-lab.com/) carried out sequencing and assembly following standard protocols. The whole genome library of genomic DNA was sequenced using the Illumina HiSeq 2500 platform (2 × 150 base paired-end reads) (Illumina, USA), which yielded ~ 1 million reads. The Illumina DNA Flex kit was used for the construction of the paired-end library with standard protocols. The trimming and filtering of the raw sequencing reads were performed by using NGS-Toolkit (Patel and Jain, 2012) to remove adapter contamination and low-quality reads with a cutoff of Phred quality scores of Q20, following previous protocols (Kumar et al., 2020). High-quality reads were assembled with Geneious 10 (Kearse et al., 2012) using the *Pirata subpiraticus* mitogenome (NC_025523) as a reference.

2.3. Spider mitochondrial extraction from transcriptome

Fifty-one Araneae transcriptomes, classified by family, were downloaded from NCBI's SRA database (https://www.ncbi.nlm.nih.gov/sra/?term =). Homologous contigs in each transcriptome sequence were extracted using the Trinity assembly program from a reference mitogenome. For this analysis, the mitogenome of *Phoneutria depilata* (obtained in this work) was used as a reference for obtaining mitogenomes from the transcriptomes of the Ctenidae and Trechaleidae families. Likewise, *Loxosceles similis* (NC_042902) was used as a reference from the transcriptomes of the Leptonetidae family, *Telamonia vlijmi* (NC_024287) was used as a reference from the transcriptomes of the Salticidae and Desidae families, *Cyriopagopus hainanus* (MN877932) was used as a reference from the transcriptomes of the Theraphosidae family and *Pardosa laura* (NC_025223) was used as a reference from the transcriptomes of the Lycosidae family. Mitogenome assembly was performed with Bowtie2 mapper using the Geneious Prime platform using a High-medium sensitivity setting. Only mitogenomes obtained with a total coverage of 70% (>250X average on depth of coverage) and 13 protein-coding genes (PCGs) coverage of 95% or more were taken into account for the analysis. The data on the transcriptomes used and the process of mitogenome extraction are summarized in Table 1.

2.4. Mitochondrial genome annotation and gene rearrangement confirmation

The annotation of PCGs and rRNAs for *P. depilata* and 17 other Araneae mitogenomes (obtained by transcriptome extraction) were initially identified via the MITOS web server (Bernt et al., 2013). The annotation of the tRNA genes was performed using tRNAscan-SE 1.21 (Lowe and Eddy, 1997). The circular image of nine Ctenidae mitogenomes was drawn by using the online server CGView (Grant and

Table 1
The data of the spider transcriptomes used and the process of mitogenome extraction. NCBI Ref ID of each mitogenome is shown as Ref-Seq in the mitochondrial ensemble proceeding. Molecular features such as base pairs (bp) size and percentage coverage of the assembled spider mitogenomes obtained in this work are shown. *Not included in phylogenetic analyses.

Family	*Species*	SRA ID	Mitogenome Ref-Seq	NCBI Ref ID	Assembled/Ref-Seq (bp)	% total coverage	13 PCGs (bp)	% PCGs coverage	selected for analysis
Theraphosidae (1)	*Aphonopelma hentzi*	SRR13605914	*Cyriopagopus hainanus*	MN877932	11167/13874	80.48	10716/10710	100.05	Yes
Leptonetidae (20)	*Appaleptoneta fiskei*	SRR13580909	*Loxosceles similis*	NC_042902	13760/14683	93.71	10674/10648	100.24	Yes
	Calileptoneta cokendolpheri	SRR13580908	*Loxosceles similis*	NC_042903	13673/14683	93.1	10611/10648	99.65	Yes
	Ozarkia georgia	SRR13580910	*Loxosceles similis*	NC_042904	12189/14683	83.01	10653/10648	100.04	Yes
	Cataleptoneta aesculapii	SRR13580897	*Loxosceles similis*	NC_042905	1809/14683	12.32	–	–	Not
	Leptoneta comasi	SRR13580898	*Loxosceles similis*	NC_042906	2150/14683	14.64	–	–	Not
	Leptonetela jiulong	SRR13580899	*Loxosceles similis*	NC_042907	1799/14683	12.25	–	–	Not
	Cataleptoneta sengleti	SRR13580901	*Loxosceles similis*	NC_042908	1901/14683	12.94	–	–	Not
	Sulcia cretica	SRR13580902	*Loxosceles similis*	NC_042909	2202/14683	14.99	–	–	Not
	Longileptoneta sp	SRR13580904	*Loxosceles similis*	NC_042910	1899/14683	12.93	–	–	Not
	Falcileptoneta sp	SRR13580907	*Loxosceles similis*	NC_042911	1780/14683	12.12	–	–	Not
	Ozarkia georgia	SRR13580910	*Loxosceles similis*	NC_042912	1699/14683	11.57	–	–	Not
	Tayshaneta fawcetti	SRR13580911	*Loxosceles similis*	NC_042913	1809/14683	12.32	–	–	Not
	Tayshaneta myopica	SRR13580914	*Loxosceles similis*	NC_042914	1809/14683	12.32	–	–	Not
	Calileptoneta helferi	SRR13580915	*Loxosceles similis*	NC_042915	1809/14683	12.32	–	–	Not
	Chisoneta modica	SRR13580917	*Loxosceles similis*	NC_042916	1901/14683	12.94	–	–	Not
	Protoleptoneta italica	SRR13580919	*Loxosceles similis*	NC_042917	2150/14683	14.64	–	–	Not
	Paraleptoneta spinimana	SRR13580920	*Loxosceles similis*	NC_042918	1454/14683	9.9	–	–	Not
	Leptoneta infuscata	SRR13580922	*Loxosceles similis*	NC_042919	2150/14683	14.64	–	–	Not
	Barusia laconica	SRR13580923	*Loxosceles similis*	NC_042920	2103/14683	14.32	–	–	Not
	Barusia maheni	SRR13580924	*Loxosceles similis*	NC_042921	2103/14683	14.32	–	–	Not
Salticidae (17)	*Bavia aericeps*	SRR12832794	*Telamonia vlijmi*	NC_024287	14270/14601	97.73	10707/10722	99.86	Yes
	Maripanthus draconis	SRR12832802	*Telamonia vlijmi*	NC_024287	14171/14601	97.04	10749/10722	100.25	Yes
	Padillothorax badut	SRR12832803	*Telamonia vlijmi*	NC_024287	13997/14601	95.86	10785/10722	100.58	Yes
	Phidippus johnsoni	SRR12832797	*Telamonia vlijmi*	NC_024287	12059/14601	82.59	10740/10722	100.16	Yes
	Piranthus planolancis	SRR12832799	*Telamonia vlijmi*	NC_024287	14592/14601	99.93	10695/10722	99.74	Yes
	Indopadilla kahariana	SRR12832791	*Telamonia vlijmi*	NC_024287	1452/14601	9.94	–	–	Not
	Bavia sexpunctata	SRR12832792	*Telamonia vlijmi*	NC_024287	3540/14601	24.24	–	–	Not
	Bavia nessagyna	SRR12832793	*Telamonia vlijmi*	NC_024287	3540/14601	24.24	–	–	Not
	Salticus scenicus	SRR12832795	*Telamonia vlijmi*	NC_024287	2711/14601	18.56	–	–	Not
	Menemerus bivittatus	SRR12832796	*Telamonia vlijmi*	NC_024287	4003/14601	27.41	–	–	Not
	Ligurra latidens	SRR12832798	*Telamonia vlijmi*	NC_024287	3062/14601	20.97	–	–	Not
	Maripanthus reinholdae	SRR12832801	*Telamonia vlijmi*	NC_024287	1990/14601	13.62	–	–	Not
	Stagetillus cf. *opaciceps*	SRR12832804	*Telamonia vlijmi*	NC_024287	6009/14601	41.15	–	–	Not
	Indopadilla redynis	SRR12832805	*Telamonia vlijmi*	NC_024287	1452/14601	9.94	–	–	Not
	Indopadilla kodagura	SRR12832806	*Telamonia vlijmi*	NC_024287	1452/14601	9.94	–	–	Not
	Helpis minitabunda	SRR12832807	*Telamonia vlijmi*	NC_024287	2711/14601	18.56	–	–	Not
	Attulus floricola	SRR12832808	*Telamonia vlijmi*	NC_024287	2711/14601	18.56	–	–	Not
Desidae (2)	*Desis marina*	SRR12968639	*Telamonia vlijmi*	NC_024287	1892/14601	12.95	–	–	Not
	Badumna longinqua	SRR12963054	*Telamonia vlijmi*	NC_024287	1892/14601	12.95	–	–	Not
Lycosidae (1)	*Schizocosa ocreata*	SRR13511132	*Pardosa laura*	NC_025223	3701/14513	25.5	–	–	Not
Trechaleidae (2)	*Cupiennius salei*	SRR880446	*P. depilata*	MZ902035	11554/14734	78.41	9297/10683	87.02	Yes
	Cupiennius coccineus	SRR7028538	*P. depilata*	MZ902035	3701/14734	25.11	–	–	Not
Ctenidae (8)	*Leptoctenus byrrhus*	SRR10413987	*P. depilata*	MZ902035	14564/14734	99.31	10679/10683	99.96	Yes
	Phoneutria nigriventer	SRR10413989	*P. depilata*	MZ902035	14618/14734	98.88	10195/10683	95.42	Yes

(*continued on next page*)

Table 1 (*continued*)

Family	*Species*	SRA ID	Mitogenome Ref-Seq	NCBI Ref ID	Assembled/Ref-Seq (bp)	% total coverage	13 PCGs (bp)	% PCGs coverage	selected for analysis
	Anahita punctulata	SRR10414003	*P. depilata*	MZ902035	12041/14734	81.72	10347/10683	96.85	Yes
	Isoctenus ordinario	SRR10413992	*P. depilata*	MZ902035	11890/14734	80.69	10275/10683	96.18	Yes
	Ctenus hibernalis	SRR10413993	*P. depilata*	MZ902035	11108/14734	75.39	10041/10683	93.99	Yes*
	Ctenus exlineae	SRR10414004	*P. depilata*	MZ902035	10862/14734	73.72	10143/10683	94.99	Yes*
	Ctenus captiosus	SRR10413998	*P. depilata*	MZ902035	10470/14734	71.06	10104/10683	94.58	Yes
	Ctenus corniger	SRR6360557	*P. depilata*	MZ902035	2418/14734	16.41	–	–	Not

Stothard, 2008) (http://stothard.afns.ualberta.ca/cgview_server/). We retrieved the sequences and gene annotations of the 58 complete spider mitogenomes that are available at the organelle genome resources database from NCBI (https://www.ncbi.nlm.nih.gov/genome/browse#!/organelles/) as of January 20, 2021.

Gene annotation verification analysis was based on three previously postulated methodologies (Prada and Boore, 2019): a) All gene annotations were refined using other spider mitochondrial sequences from GenBank using the program Muscle (Edgar, 2004) with Geneious 10 (Kearse et al., 2012) to confirm the locations and boundaries of each gene. Nucleotide identity greater than 80% was used to confirm the orientation of each gene with possible rearrangement. b) The NCBI-BLAST2 sequence comparison program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to confirm rearrangements in the spider mitogenome by comparing nucleotide sequences of orthologous regions from evolutionarily close species. c) Putative tRNA inversions were verified by tRNAscan-SE 1.21. The orientation of tRNAs was taken as positive when it coincides in two of the three methodologies used.

After confirming each genetic annotation, numerical gene order was determined (from 1 to 38, considering 1 as the *trnM* and 2 as *nad2* gene; and so on) and their gene orientation (plus/plus as + plus/minus as −). A list of spider species, arranged taxonomically and provided with their GenBank IDs, reported gene rearrangements (color-coded to the results of these analyses), of each mitochondrial gene and genome, as annotated in these GenBank records, is given in Supplementary Table S1.

The D-loop regions were extracted, and their number of base pairs (bp) and CG and AT contents were identified using Geneious Prime (Kearse et al., 2012). To calculate the skewness, we used the formula AT skew=(A − T)/(A + T) and GC skew=(G − C)/(G + C) (Perna and Kocher, 1995). Multiple alignment was performed by the Clustal Omega program (Sievers and Higgins, 2021). Inverted repeats or palindromes in the D-loop region were checked using Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.html).

2.5. Phylogenetic analysis

A total of 58 Araneae mitogenomes available in GenBank and 18 obtained in this work were included in the phylogenetic analysis. The complete scorpion mitogenome *Buthus occitanus* (NC_010765) was selected as an outgroup of Araneae (Supplementary Table S1), because of the few scorpion mitogenomes sequenced, their gene order representative of the family Buthidae (Scorpiones) is closest to that observed in spiders of the family Liphistiidae (Ban et al. 2022). The 13 PCGs of all 75 spiders and the out-group were aligned with the MAFFT program (Katoh and Standley, 2013) and subsequently concatenated (the gene order in plus/plus orientation is as follows: *atp6, atp8, cox1, cox2, cox3, cob, nad1, nad2, nad3, nad4, nad4 L, nad5 and nad6*). The phylogenetic tree of Kulkarni et al. (2021), based on the analysis of ultraconserved elements (UCEs) was used as to to construct a backbone constraint tree in our phylogenetic analysis. Substitution models were explored using ModelFinder implemented in IQTREE (Nguyen, 2015; Kalyaanamoorthy, 2017) using the corrected Akaike information criterion (AICc). ML analysis was made using IQ-tree (Nguyen et al., 2015) implemented on CIPRES platform (https://www.phylo.org/). The robustness of the maximum likelihood tree topology was tested by 1000 bootstrap pseudoreplicates of the tree search. The phylogenetic tree was visualized and edited using FigTree v1. 4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

2.6. Gene arrangement analysis

Common interval analysis was performed using CREx (Bernt et al., 2007) for pairwise comparisons for inference of ancestral genome states. Pairwise comparisons were conducted for each spider mitogenome against the scorpion (*Buthus occitanus*) mitochondrial genome to determine the minimum number of genome rearrangement events separating each genic order from the inferred ancestral state.

3. Results and discussion

3.1. Mitogenome features

The complete mitogenome sequences of nine Ctenidae species range from 13,035 bp in *Ctenus hibernalis* to 14,724 bp in *Phoneutria depilata*. Comparison of the genetic characteristics and molecular composition of the mitogenome of *P. depilata* with the other mitogenomes obtained in this work are summarized in Table 2 and supplementary table S1.

Likewise, from the 51 transcriptomes analyzed in this work, only 18 mitogenomes satisfied our inclusion criteria; in some of them, small gaps in the previously mentioned mitochondrial regions were found (Table 1). The map of the eight Ctenid mitogenomes is summarized in Fig. 1. This size variation was due to the process of extraction and assembly from transcriptomes available in databases. For example, in Ctenidae species, our results show the nucleotide sequence absence of several tRNAs, identifying between 9 and 20 genes (out of a total of 22 tRNAs). In addition, the absence of the sequence corresponding to the *nad4 L* gene (in *Ctenus hibernalis,* and *Ctenus exlineae* and the *nad3* gene (*Anahita punctulata*) was also detected. Similar molecular features were observed in *Cupiennius salei* corresponding to Trechaleidae family.

According to Forni et al. (Forni et al., 2019), although the procedure to reconstruct complete mitogenomes from RNA-Seq sequence reads is efficient, certain mitochondrial regions, such as tRNAs, D-loops and a fragment of the *atp6* gene, have low read representation and therefore often generate small gaps during the assembly process. In this same work, the authors indicate that the percentage of reads reached during the process of extraction and assembly of mitogenomes from transcriptomes varies from 16 to 57% using both intrafamilial and congeneric starting references, filtering for possible contaminants and nuclear gene matches (Forni et al., 2019).

Previous studies showed that, in addition to the low coverage (<4x) in the aforementioned mitochondrial regions, other characteristics of the RNA-Seq sequence data (such as depth of coverage or platform type)

Table 2
Features of the eighteen spider mitochondrial genomes obtained in this work. *The total size in bp was calculated based on the reference genome and the missing gaps in the sequence were filled with N.

Family	Species	Size (bp)*	%CG	Annotated genes (%CG)			
				PCGs	tRNA	rRNA	D-loop
Theraphosidae	*Aphonopelma hentzi*	12,991	31.3	13 (31.5)	22 (30.6)	2 (30.5)	0
Leptonetidae	*Appaleptoneta fiskei*	13,945	33.4	13 (34.9)	22 (30.1)	2 (27.1)	1 (30.2)
	Calileptoneta cokendolpheri	14,022	34.7	13 (36.3)	22 (28.1)	2 (29.5)	1 (34.5)
	Ozarkia georgia	13,654	30.3	13 (30.4)	22 (26.9)	2 (29.2)	0
Salticidae	*Bavia aericeps*	14,270	22.7	13 (23.4)	22 (22.4)	2 (19.1)	1 (18.6)
	Maripanthus draconis	14,371	24.1	13 (25.2)	22 (24.4)	2 (19.5)	1 (17.2)
	Padillothorax badut	14,058	23.0	13 (23.6)	22 (21.5)	2 (20.0)	1 (21.1)
	Phidippus johnsoni	14,749	23.6	13 (24.2)	22 (22.7)	2 (20.0)	1 (19.9)
	Piranthus planolancis	14,592	24.2	13 (25.2)	22 (22.4)	2 (18.7)	1 (21.2)
Trechaleidae	*Cupiennius salei*	13,826	21.6	12 (22.3)	17 (21.8)	2 (18.9)	0
Ctenidae	*Anahita punctulata*	14,022	25.1	12 (25.9)	10 (21.8)	2 (21.6)	1 (24.4)
	Ctenus hibernalis	13,035	25.9	12 (26.6)	9 (27.1)	2 (21.8)	0
	Ctenus captiosus	13,110	24.9	13 (24.9)	14 (28.9)	2 (22.3)	0
	Ctenus exlineae	13,505	26.8	12 (27.6)	10 (30.4)	2 (21.5)	0
	Isoctenus ordinario	13,434	24.5	13 (25.1)	14 (24.6)	2 (20.3)	0
	Leptoctenus byrrhus	14,192	27.7	13 (29.3)	20 (24.8)	2 (21.0)	1 (25.7)
	Phoneutria depilata	14,724	28.4	13 (29.7)	22 (25.6)	2 (22.6)	1 (28.3)
	Phoneutria nigriventer	14,186	26.3	13 (27.5)	19 (24.5)	2 (21.3)	1 (24.8)

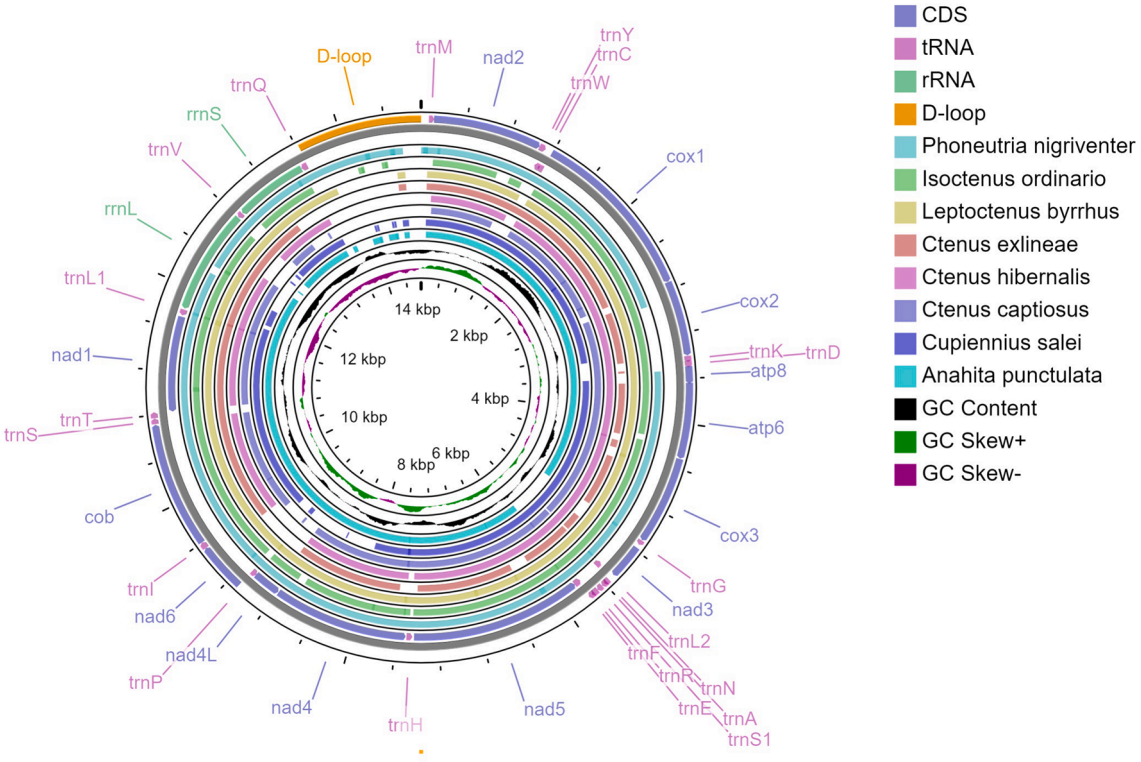

Fig. 1. Circular representation of the complete mitogenome of nine Ctenidae spiders. The direction of 37 genes (PCGs, tRNAs and rRNAs) and the Dloop region are indicated by arrows in the entire complete genome. PCGs are shown as purple arrows, tRNA genes as pink color arrows, rRNA genes as green arrows, and Dloop regions as orange rectangles. The GC content is plotted using a black sliding window. The GC skew is plotted using a colored sliding window (green and orchid color) as the deviation from the average GC skew of the entire sequence. The figure was made using CGView online server (http://stothard.afns.ualberta.ca/cgview_server/). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

or the use of reference mitogenomes (closest evolutionary) are determining factors in obtaining the most complete mitogenome possible (Moreira et al., 2015; Forni et al., 2019).

Nine spider mitogenomes of an additional three families were obtained in this work, ranging in size from 12,991 bp (*Aphonopelma hentzi*, Theraphosidae) to 14,749 bp (*Phidippus johnsoni,* Salticidae) (Supplementary Table S1). The difference in size between these mitogenomes is mainly due to the absence of the D-loop region sequence not only in those obtained in this work but also in those deposited in the databases. Despite this, the relatively small mitogenome sizes (less than15 kb) are well within the observed range of other Araneae mitogenomes available from the GenBank database (Fang et al., 2016; Wang et al., 2016; Zhu and Zhang, 2017; Kumar et al., 2020; Yong et al., 2021) compared to those of other Arthropod species (Cameron, 2014). In addition, truncated tRNA genes may explain why spider mitogenomes are smaller than those of other arthropods (Pons et al., 2019)

The GC content of nucleotides was between 21.6% (*Cupiennius salei*, Trechaleidae) and 34.7% (*Calileptoneta cokendolpheri*, Leptonetidae).

The highest GC content was observed in the PCG (22.3–36.3%), followed by tRNAs (21.8–30.6%), rRNAs (18.9–30.5%), and D-loops (17.2–34.5%). All eighteen spider mitogenomes presented the structure of 37 genes (13 PCGs, 22 tRNAs and two rRNAs) and an A + T-rich D-loop region, where a total of 573 genes were annotated. However, some of these species did not have this complete gene set. The features of the eighteen spider mitogenomes obtained in this work are summarized in Table 2 and Supplementary Table S1.

As in most Araneae mitogenomes, the nucleotide composition shows a low G + C, with contents of less than 35% and small variations between species (Wang et al., 2016; Yong et al., 2021), similar to those observed in Metazoan mitogenomes (Hassanin et al., 2005). In addition, the majority strand contains 22 genes and a minority contains 15 genes, similar features to those presented in other spider mitogenomes (Kumar et al., 2020; Yong et al., 2021).

3.2. Variation in the D-loop region in spiders

The D-loop region was detected and annotated in 11 of the 18 mitogenomes obtained in this work. Similarly, this region was observed in 66 of the 76 spiders, located between *trnM* and *trnQ* in all Opisthothelae spiders and between *rrnS* and *trnI* in Mesothelae spiders (Supplementary Table S1). The position of the D-loop region is consistent with that observed in previous work in several spider species (Kumar et al., 2020; Yong et al., 2021). In addition, previous studies indicate that the D-loop region remained totally or partially assembled, mainly because it is composed of tandem repeats, which are very difficult to sequence and *de novo* assemble properly; likewise, their expression in RNA-Seq experiments is low or completely lacking (Forni et al., 2019). Moreover, we obtained complete or partial D-loop regions in 61% of them when mitogenomes of congeneric species were used as reference.

Our results show that the D-loop of the 66-spider region is characterized by an average size of 766 bp, an average AT % of 75.1% and a low nucleotide identity of 18.7%, showing high variation in this species (Supplementary Table S3). For example, the two Mesothelae (Liphistiidae species) and the five Mygalomorphae species (Nemesiidae (1), Theraphosidae (3), Dipluridae (1)) have a D-loop region with an average of 388 bp, while the Araneomorphae species have a D-loop region with an average of 800 bp. The largest D-loop region was observed in *Argyroneta aquatica* (Cybaeidae) at 2047 bp, where both 5′ and 3′ AT tandem repeat expansions were detected. Likewise, the AT content was variable in all species, ranging from 66.3% to 82.6% (Supplementary Table S2). However, most of the D-loop regions of Liphistiidae, Theraphosidae, Dipluridae, Dysderidae, Hypochilidae, Sicariidae, Pholcidae, Oecobiidae, Araneidae, Eutichuridae and Selenopidae showed positive AT/GC skews, indicating an obvious bias toward the use of A and G, similar to those reported in *Lyrognathus crotalus* (Theraphosidae (Kumar et al., 2020) and other spiders (Pons et al., 2019). In contrast, our analyses show that mainly Entelegynae spiders have a negative AT/GC skew, indicating a bias toward the use of T and G.

Regarding the presence of repetitive regions, our results show three categories of D-loop regions: a) sequences with a size in bp of $\leq$ 500 bp (n = 14) that do not have tandem repeat elements; b) D-loop regions between 500 and 740 bp (n = 25), that do not have tandem repeats or that are of low complexity (AT-rich regions with less than 20 nucleotides and less than 2 copies); and c) D-loop regions above 740 bp (n = 27), that have tandem repeats of higher complexity (AT-rich regions with more than 20 nucleotides and more than 3 copies). The molecular characteristics of the D-loop region in the spider species analyzed are summarized in Supplementary Table S2.

Although variation in these molecular features (AT/GC skew and presence of tandem repeat elements) seem to differentiate Synspermiata from Entelegynae, a larger number of complete D-loop regions needs to be analyzed to corroborate this hypothesis.

3.3. Gene annotation errors and rearrangement level in the Araneae mitogenome

According to the annotation of the 58 spider mitogenomes found in the NCBI database, there were a total of 203 cases of 2,166 genes annotated to be in arrangements differing from the gene order of the scorpion mitogenome *Buthus occitanus*. Of all these reorganizations, 17 were identified as gene annotation errors, which means an error rate of 8.4% (17/203), indicating that 21% (12/57) of mitogenomes have at least one gene mistakenly annotated (Supplementary Table S1). For example, the mitogenome with the highest number of annotation errors (5) was that of *Amaurobius fenestralis* (Amaurobiidae), in which the order *trnS, trnR, trnE, -trnL* and *-trnF,* without annotation of *trnA* and *trnN* genes, was reported. Our analysis indicates that the *-trnL2, trnN, trnA, trnS1, trnR, trnE* and *-trnF* genes are found in this region, confirming the annotation error. In addition to false inversions, our analyses indicate a high number (40) of unannotated genes, but our results confirm their presence position in the genome. Most of these unannotated genes were tRNAs (21), followed by the D-loop region (19) (Supplementary Table S1). Examples of false inversions and deletions in the spider mitogenome are shown in Supplementary Figure S1.

Previously, it has been reported that thousands of mitogenomes deposited in the database have shown a high rate of annotation errors, most of which are easily detectable (Boore, 2006; Donath et al., 2019). Earlier studies indicate that such errors are frequent in the Hexapoda mitogenomes, with an annotation error rate of 5.5% concentrated mainly in tRNA genes (Moreno-Carmona et al., 2021). Although our results confirm that annotation errors in the spider mitogenome are concentrated in tRNAs, the error rate is almost double of that observed in Hexapoda, generating false gene orders in this taxonomic group.

3.4. Phylogenetic analysis

We reconstructed phylogenomic trees based on the mitogenomes of 76 spider species and an outgroup species based on the nucleotide sequence of 13 mitochondrial protein-coding genes using maximum likelihood (ML) (Fig. 2). In this phylogenetic reconstruction, the Araneae species follow the same backbone topology of the tree of Kulkarni et al. (2021); where the monophyly of Araneae was confirmed in the tree. Our findings provide strong support for the monophyly of Opisthothelae and Mesothelae with high supporting values. In addition, the subdivision of Opisthothelae into two monophyletic groups, Mygalomorphae and Araneomorphae infraorders, was supported in the phylogenetic tree; however, Araneomorphae species are grouped in a monophyletic group. The estimated tree in this study also supports the monophyly of Synspermiata and Entelegynae. For example, within Entelegynae, there is a monophyletic group composed of the species of the families Tetragnhatidae and Araneidae, which represent the Araneoidea clade. Similarly, Uroctea compactilis (Oecobiidae, UDOH grade) is sister to the RTA clade. Likewise, the estimated ML tree from the mitogenome is consistent with those observed in previous analyses of 13 PCG mitochondrial genes (Pons et al., 2019; Kumar et al., 2020) and a multilocus phylogeny (Wheeler et al., 2017a). However, more mitogenome data of Mesothelae, Mygalomorphae and Aranemorphae taxa would cover the consistent sign of deep phylogenetic relationship between all Araneae species.

In addition to including new spider mitogenomes from different Araneae species, the phylogenetic relationships of the family Ctenidae were evaluated. In this case, *Isoctenus ordinarius* is ancestral sequence of the family Ctenidae as monophyletic group; where the mitogenome sequence of *Phoneutria depilata* clustered with *P. fera*, forming a monophyletic clade and paraphyletics of *Anahita punctulata*. Likewise, *Leptoctenus byrrhus* and the species of the genus *Ctenus* form a monophyletic group, sister to the genus Phoneutria (Fig. 2). after the dot, include the following sentence: Our P. depilata mitogenome was placed within the family Ctenidae closely related to P. nirgiventer with high support. The

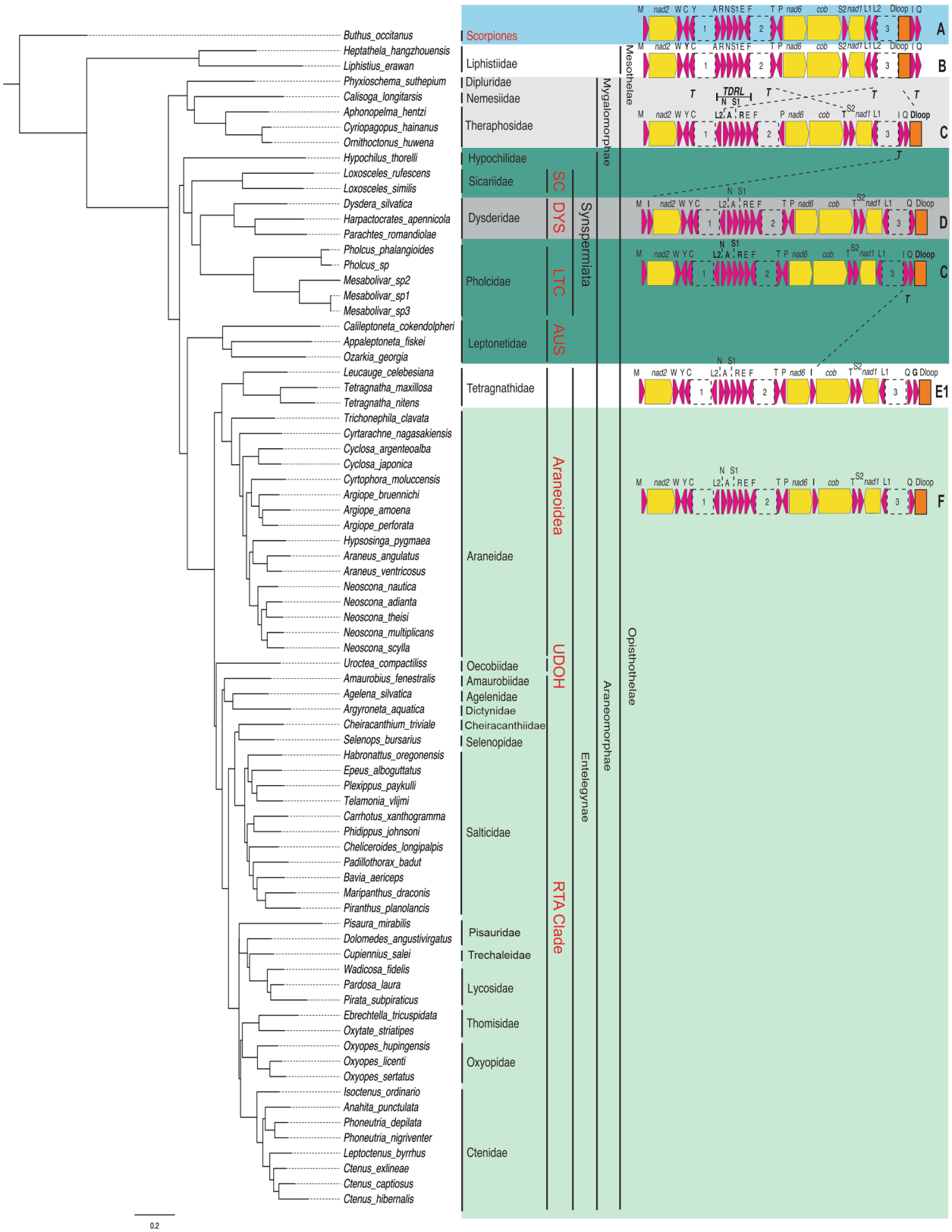

Fig. 2. Maximum likelihood phylogenetic tree inferred by 13 PCGs and CREX analyses of the 76 Araneae mitogenomes. The complete scorpion mitogenome *Buthus occitanus* (NC_010765) was selected as an outgroup. The phylogenetic tree was visualized and edited using FigTree v1. 4.2 (http://tree.bio.ed.ac.uk/software/figtree/). In the CREX analyses (right), PCGs are shown as yellow arrows, tRNA genes as purple color arrows, and D-loop regions as orange rectangles. The dotted boxes with numbers represent conserved blocks of genes: 1 (*cox1-cox2-trnK-trnD-atp8-atp6-cox3-trnG-nad3*), 2 (*nad5-trnH-nad4-nad4 L*) and 3 (*rrnL-trnV-rrnS*). The letters **T** are shown as translocation events, and the **TDRL** is shown as tandem duplication and random loss events. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

family Ctenidae was a sister group of a clade comprising the families Oxyopidae and Thomisidae with high support. Although our study has few representatives of Ctenidae, the phylogenetic relationships obtained using only mitogenomes were congruent with a recent multilocus phylogeny of Ctenidae (Hazzi and Hormiga, 2022)

3.5. Spider mitogenome architectures and CREX analysis

By comparing the mitochondrial gene order rearrangement scenario in Araneae with the scorpions (*Buthus occitanus*) inferred by CREx analysis, three tRNAs (*trnY*, *trnL2*, and *trnI*) and a Dloop region were found to transposition and one TDRL event in block *trnN-trnA-trnS1-trnR* (Fig. 2). According to Ban et al., (2022), the genetic order in *Buthus occitanus* is conserved throughout the family Buthidae as well as in other scorpion species. However, the low number of species analyzed (8 mitogenomes available so far) could lead to a bias in identifying the ancestral order of all scorpions.

Compared to the putative gene order scorpion architecture of *Buthus occitanus* (A), \ Mesothelae ("primitive spiders") present only one translocation of the *trnY* gene in the *trnW-trnY-trnC* region (architecture B). Mygalomorphae and Synspermiata species differ from Mesothelae by a tandem duplication and random loss (TDRL) event in the *trnN-trnA-trnS1-trnR* region, a *trnL2* gene translocation event in the same region, a *trnT* gene translocation event in the *cob-trnS2* region and a translocation of the D-loop region (architecture C). Only the three Dysderidae species differ from Hypochilidae, Sicaridae, Pholcidae and Leptonetidae families by translocation of the *trnI* gene in the *trnM-nad2* region (architecture D). In addition, all Entelegynae differ from most Synspermiata (architecture C) by translocation of the *trnI* gene in the *nad6-cob* region (architecture E and F). However, two of the three Tetragnathidae species have a different architecture. *Tetragnatha maxillosa* varies in architecture F by translocation of the *trnG* gene in the *trnQ-D loop* region (architecture E1). In addition, *Tetragnatha nitens* (architecture E2) has a new translocation of the *trnW* gene in the *trnQ-Dloop* region, evidencing a generic order trnQ-*trnW-trnG-Dloop*. Our analysis indicates that *Leucauge celebesiana* has the gene order of architecture F (Supplementary table S1 and Fig. 2).

Mitogenomes have been recently used to study the phylogeny and evolution of spiders (Tyagi et al., 2020; Yong et al., 2021). The different gene orders or architectures observed in spider mitogenomes available in public databases, displaying an accelerated gene rearrangement in Araneomorphae compared to Mygalomorphae (Wang et al., 2016; Pons et al., 2019; Kumar et al., 2020; Tyagi et al., 2020). However, despite the low number of species analyzed, these gene rearrangements are overrepresented due to the large number of annotation errors, generating up to nineteen mitochondrial gene rearrangement types and 12 architectures among 44 spiders (Tyagi et al., 2020), while in our analysis of 76 spider species, nine mitochondrial gene rearrangement types (8 translocation and one TDRL event) and six architectures were confirmed (see Supplementary Table S1 and Fig. 2).

4. Conclusion

The complete mitogenome of *Phoneutria depilata* was characterized, which allowed the extraction from transcriptomes of an additional seven ctenid mitogenomes, as well as ten other species of different families and comparison with other members of Araneae. The analysis of the spider mitogenomes available in the database shows that a significant rate of gene annotation error in this taxonomic group significantly, overexpressing the rate of gene rearrangement in this group. In addition to expanding the number of s available spiders mitogenomes, our analyses report a phylogenetic position of the families Ctenidae and Leptonetidae, which provides a potentially more robust phylogeny and systematics of Araneae. Most importantly, the findings from this study provide new evidence of a low rate of rearrangement among spider mitogenomes, with divergences observed at the phylogenetic and genetic levels between Mesothelae and Opisthothelae spiders.

Funding

We thank the University of Tolima for supporting this research. The first author thanks Oficina de Investigaciones y Desarrollo Científico de la Universidad del Tolima for postdoctoral fellowships (4/2019). We are grateful to the Departamento Administrativo de Ciencia, Tecnología e Innovación (Colciencias-currently Minciencias, Colombia) for financial support for this project (ID Project 130780864623). GH acknowledges support form US National Science Foundation Grant 1754262.

CRediT authorship contribution statement

Carlos F. Prada: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Nicolas A. Hazzi:** Writing – review & editing. **Gustavo Hormiga:** Writing – review & editing. **Felipe Cabarcas:** Methodology, Validation, Writing – original draft, Writing – review & editing. **Lida M. Franco:** Methodology, Validation, Writing – original draft, Writing – review & editing.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2022.146925.

References

Adrián-Serrano, S., Lozano-Fernandez, J., Pons, J., Rozas, J., Arnedo, M.A., 2021 Jan. On the shoulder of giants: mitogenome recovery from non-targeted genome projects for phylogenetic inference and molecular evolution studies. J. Zool. Syst. Evolut. Res. 59 (1), 5–30.

Ban, X.C., Shao, Z.K., Wu, L.J., Sun, J.T., Xue, X.F., 2022. Highly diversified mitochondrial genomes provide new evidence for interordinal relationships in the Arachnida. Cladistics.

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., Pütz, J., Middendorf, M., Stadler, P.F., 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol. Phylogenet. Evol. 69, 313–319.

Bernt, M., Merkle, D., Ramsch, K., Fritzsch, G., Perseke, M., Bernhard, D., Schlegel, M., Stadler, P.F., Middendorf, M., 2007. CREx: inferring genomic rearrangements based on common intervals. Bioinformatics 23, 2957–2958.

Boore, J.L., 1999. Animal mitochondrial genomes. Nucleic Acids Res. 27, 1767–1780.

Boore, J.L., 2006. Requirements and standards for organelle genome databases. Omics 10, 119–126.

Bucaretchi, F., Bertani, R., De Capitani, E.M. and Hyslop, S.J.C.T., 2016. Envenomation by wandering spiders (genus Phoneutria). 63, 1-49.

Cameron, S.L., 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. Annu Rev Entomol 59, 95–117.

Catalog, W.S., 2022. World spider catalog. Version 23.5. Natural History Museum Bern. online at http://wsc.nmbe.ch, accessed on feb 2022. doi: 10.24436/2.

Cathrine, C. and Longhorn, S.J.N.o.t.B.A.S., 2017. Record of Phoneutria (Araneae: Ctenidae) from Inverbervie, Aberdeenshire. Newsletter of the British Arachnological Society. 139, 13-15.

Donath, A., Jühling, F., Al-Arab, M., Bernhart, S.H., Reinhardt, F., Stadler, P.F., Middendorf, M., Bernt, M., 2019. Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. Nucleic Acids Res. 47, 10543–10552.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Estrada-Gomez, S., Vargas Munoz, L.J., Lanchero, P. and Segura Latorre, C.J.T., 2015. Partial characterization of venom from the Colombian spider Phoneutria boliviensis (Aranae: Ctenidae). 7, 2872-2887.

Fang, W.Y., Wang, Z.L., Li, C., Yang, X.Q., Yu, X.P., 2016. The complete mitogenome of a jumping spider Carrhotus xanthogramma (Araneae: Salticidae) and comparative analysis in four salticid mitogenomes. Genetica 144, 699–709.

Florez, E., Ortiz, A. and Montoya, M.J.E., 2003. Accidentes por mordedura de la araña de las bananeras Phoneutria boliviensis (Araneae: Ctenidae) en la región de Urabá, Colombia. Entomólogo, 96(31), 1-4.
Foelix, R., 2010. Biology of spiders, (3rd edn.) ed. Oxford University Press, New York.
Forni, G., Puccio, G., Bourguignon, T., Evans, T., Mantovani, B., Rota-Stabelli, O., Luchetti, A., 2019. Complete mitochondrial genomes from transcriptomes: assessing pros and cons of data mining for assembling new mitogenomes. Sci. Rep. 9, 14806.
Gissi, C., Iannelli, F., Pesole, G., 2008. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity (Edinb) 101, 301–320.
Grant, J.R., Stothard, P., 2008. The CGView Server: a comparative genomics tool for circular genomes. Nucleic Acids Res. 36, W181–W184.
Hassanin, A., Léger, N., Deutsch, J., 2005. Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of metazoa, and consequences for phylogenetic inferences. Syst. Biol. 54, 277–298.
Hazzi, N.A. and Hormiga, G., 2021. Corrigenda: Morphological and molecular evidence support the taxonomic separation of the medically important Neotropical spiders Phoneutria depilata (Strand, 1909) and P. boliviensis (F.O. Pickard-Cambridge, 1897) (Araneae, Ctenidae). ZooKeys 1022: 13-50. https://doi.org/10.3897/zookeys.1022.60571. Zookeys 1033, 203-205.
Hazzi, N. A., & Hormiga, G. (2022). Molecular phylogeny of the tropical wandering spiders (Araneae, Ctenidae) and the evolution of eye conformation in the RTA clade. Cladistics. https://doi.org/10.1111/cla.12518.
Hazzi, N.A., Valderrama-Ardila, C., Brescovit, A.D., Polotow, D., Simó, M., 2013. New records and geographical distribution of ctenid spiders (Araneae: Ctenidae) in Colombia. Zootaxa 3709, 243–254.
Hazzi, N.A.J.T.J.o.A., 2014. Natural history of Phoneutria boliviensis (Araneae: Ctenidae): habitats, reproductive behavior, postembryonic development and prey-wrapping. 42, 303-310.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.
Kulkarni, S., Kallal, R.J., Wood, H., Dimitrov, D., Giribet, G., Hormiga, G., 2021. Interrogating Genomic-Scale Data to Resolve Recalcitrant Nodes in the Spider Tree of Life. Mol. Biol. Evol. 38, 891–903.
Kumar, V., Tyagi, K., Chakraborty, R., Prasad, P., Kundu, S., Tyagi, I., Chandra, K., 2020. The Complete Mitochondrial Genome of endemic giant tarantula, Lyrognathus crotalus (Araneae: Theraphosidae) and comparative analysis. Sci. Rep. 10, 74.
Li, Y.T., Xin, Z.Z., Tang, Y.Y., Yang, T.T., Tang, B.P., Sun, Y., Zhang, D.Z., Zhou, C.L., Liu, Q.N., Yu, X.M., 2020. Comparative Mitochondrial Genome Analyses of Sesarmid and Other Brachyuran Crabs Reveal Gene Rearrangements and Phylogeny. Front. Genet. 11, 536640.
Lowe, T.M., Eddy, S.R., 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25, 955–964.
Lüddecke, T., Herzig, V., von Reumont, B.M., Vilcinskas, A., 2022. The biology and evolution of spider venoms. Biol. Rev. Camb. Philos. Soc. 97, 163–178.
Martins, R., Bertani, R.J.Z., 2007. The non-Amazonian species of the Brazilian wandering spiders of the genus Phoneutria Perty, 1833 (Araneae: Ctenidae), with the description of a new species. 1526, 1-36.
Masta, S.E., Boore, J.L., 2008. Parallel evolution of truncated transfer RNA genes in arachnid mitochondrial genomes. Mol Biol Evol 25, 949–959.
Moreira, D.A., Furtado, C., Parente, T.E., 2015. The use of transcriptomic next-generation sequencing data to assemble mitochondrial genomes of Ancistrus spp. (Loricariidae). Gene 573, 171–175.
Moreno-Carmona, M., Cameron, S.L., Prada Quiroga, C.F., 2021. How are the mitochondrial genomes reorganized in Hexapoda? Differential evolution and the first report of convergences within Hexapoda. Gene 791, 145719.
Nguyen, L.T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol 32, 268–274.
Patel, R.K., Jain, M., 2012. NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLoS One 7, e30619.
Perna, N.T., Kocher, T.D., 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J. Mol. Evol. 41, 353–358.
Pons, J., Bover, P., Bidegaray-Batista, L., Arnedo, M.A., 2019. Arm-less mitochondrial tRNAs conserved for over 30 millions of years in spiders. BMC Genomics 20, 665.
Prada, C.F., Boore, J.L., 2019. Gene annotation errors are common in the mammalian mitochondrial genomes database. BMC Genomics 20, 73.
Rozwałka, R., Rutkowski, T. and Bielak-Bielecki, P., 2017. New data on introduced and rare synanthropic spider species (Arachnida: Araneae) in Poland (II), Annales Universitatis Mariae Curie-Sklodowska, sectio C–Biologia. pp. 59.
Sierra Ramírez, D., Guevara, G., Franco Pérez, L.M., van der Meijden, A., González-Gómez, J.C., Carlos Valenzuela-Rojas, J., Prada Quiroga, C.F., 2021. Deciphering the diet of a wandering spider (Phoneutria boliviensis; Araneae: Ctenidae) by DNA metabarcoding of gut contents. Ecol. Evol. 11, 5950–5965.
Sievers, F., Higgins, D.G., 2021. The Clustal Omega Multiple Alignment Package. Methods Mol. Biol. 2231, 3–16.
Simó, M., Brescovit, A.-D., 2001. Revision and cladistic analysis of the Neotropical spider genus Phoneutria Perty, 1833 (Araneae, Ctenidae), with notes on related Cteninae. Bulletin-British Arachnological Society 12 (2), 67–82.
Trejos, A., Trejos, R., Zeledón, R., 1971. Aracnidismo por Phoneutria en Costa Rica (Araneae: Ctenidae). Revista de biologia Tropical 19 (1–2), 241–249.
Tyagi, K., Kumar, V., Poddar, N., Prasad, P., Tyagi, I., Kundu, S., Chandra, K., 2020. The gene arrangement and phylogeny using mitochondrial genomes in spiders (Arachnida: Araneae). Int. J. Biol. Macromol. 146, 488–496.
Valenzuela-Rojas, J.C., González-Gómez, J.C., Guevara, G., Franco, L.M., Reinoso-Flórez, G., García, L.F., 2020 Jun. Notes on the feeding habits of a wandering spider, Phoneutria boliviensis (Arachnida: Ctenidae). J. Arachnol. 48 (1), 43–48.
Valenzuela-Rojas, J.C., González-Gómez, J.C., Van der Meijden, A., Cortés, J.N., Guevara, G., Franco, L.M., Pekár, S., García, L.F.J.T., 2019. Prey and venom efficacy of male and female wandering spider. Phoneutria boliviensis (Araneae: Ctenidae) 11, 622.
Valerio, C.E., 1983. The presence of Phoneutria boliviensis (FOP Cambridge)(Araneae, Ctenidae) in Costa Rica. J. Arachnol.
Wang, Z.L., Li, C., Fang, W.Y., Yu, X.P., 2016. The Complete Mitochondrial Genome of two Tetragnatha Spiders (Araneae: Tetragnathidae): Severe Truncation of tRNAs and Novel Gene Rearrangements in Araneae. Int. J. Biol. Sci. 12, 109–119.
Wheeler, W.C., Coddington, J.A., Crowley, L.M., Dimitrov, D., Goloboff, P.A., Griswold, C.E., Hormiga, G., Prendini, L., Ramírez, M.J., Sierwald, P., 2017a. The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. Cladistics 33, 574–616.
Wheeler, W.C., Coddington, J.A., Crowley, L.M., Dimitrov, D., Goloboff, P.A., Griswold, C.E., Hormiga, G., Prendini, L., Ramírez, M.J., Sierwald, P., Almeida-Silva, L., Alvarez-Padilla, F., Arnedo, M.A., Benavides Silva, L.R., Benjamin, S.P., Bond, J.E., Grismado, C.J., Hasan, E., Hedin, M., Izquierdo, M.A., Labarque, F.M., Ledford, J., Lopardo, L., Maddison, W.P., Miller, J.A., Piacentini, L.N., Platnick, N.I., Polotow, D., Silva-Dávila, D., Scharff, N., Szűts, T., Ubick, D., Vink, C.J., Wood, H.M., Zhang, J., 2017b. The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. Cladistics 33, 574–616.
World spider catalog. 2021. Version 22.0. https://wsc.nmbe.ch/.
Yong, H.S., Song, S.L., Chua, K.O., Wayan Suana, I., Eamsobhana, P., Tan, J., Lim, P.E., Chan, K.G., 2021. Complete mitochondrial genomes and phylogenetic relationships of the genera Nephila and Trichonephila (Araneae, Araneoidea). Sci. Rep. 11, 10680.
Zhu, X.L., Zhang, Z.S., 2017. The complete mitochondrial genome of Agelena silvatica (Araneae: Agelenidae). Mitochondrial DNA B Resour. 2, 58–59.