



# Comparative analysis of mitochondrial genomes from Buthidae (Scorpiones): gene rearrangement and phylogenetic implications

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<https://zoobank.org/FC437596-ECD5-455F-81FB-21244EC9DC97>

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**Received** 28 October 2024

**Accepted** 11 December 2024

**Published** 23 January 2025

**Academic Editors** Lorenzo Prendini, Klaus-Dieter Klass

**Citation:** Xu W, Zhang G, Xu T, He K, Wang J, Liu Z, Liu H (2025) Comparative analysis of mitochondrial genomes from Buthidae (Scorpiones): gene rearrangement and phylogenetic implications. *Arthropod Systematics & Phylogeny* 83: 3–13. <https://doi.org/10.3897/asp.83.e140421>

## Abstract

Scorpions, a diverse group of arachnids consisting of over 2,000 valid species, have received limited research attention in terms of their complete mitochondrial genomes (mitogenomes). To increase the taxonomic sampling frequency of species available for study based on mitogenomes, we reconstructed the complete mitogenomes of five scorpions, *Androctonus amoreuxi* (Audouin, 1826), *Hottentotta tamulus* (Fabricius, 1798), *Leiurus quinquestriatus* (Ehrenberg, 1828), *Lychas mucronatus* (Fabricius, 1798), and *Teruelius flavopiceus* (Kraepelin, 1900) within the family *Buthidae*. These five mitogenomes had a typical circular structure, with total sizes ranging from 14,504 to 15,083 bp. Nucleotide composition analysis indicated that the sequences were biased toward A and T. The Ka/Ks ratios within 13 protein-coding genes (PCGs) were lower than 1, suggesting that they had been subject to purifying selection in *Buthidae*. Our analyses provide additional evidence on that, in scorpions, the majority of mitogenome rearrangements occurred in tRNAs. Moreover, the genes *tRNA-Asp*, *tRNA-Gln* and *tRNA-Ile* were the hotspots of rearrangement in this order. Phylogenetic analyses based on PCGs supported taxonomic relationships in this taxon. Our results might provide useful insights into the gene arrangement features of scorpion mitogenomes and lay the foundation for further studies on the family *Buthidae*.

## Keywords

Buthidae, mitochondrial genome, tRNA, phylogenetic analysis, rearrangement

## 1. Introduction

In eumetazoans, the circular mitochondrial genome (mitogenome, usually 15 to 18 kb) typically contains 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and a long

noncoding region (CR) (Boore 1999). Genes have arrangements on this highly compact genome that exhibit a notable degree of stability across extensive spans of evolutionary time (Beckenbach 2012). However, in inverte-

**Table 1.** The mitogenomes of Buthidae, Chactidae, Scorpionidae, Scorpipidae, Vaejovidae, and Salticidae used in this study.

Order	Family	Species	Size (bp)	GenBank No.
Scorpiones	Buthidae	<i>Androctonus amoreuxi</i>	15,064	PP778689
		<i>Buthus occitanus</i>	15,060	EU523755.1
		<i>Centruroides limpidus</i>	14,519	AY803353.1
		<i>Centruroides vittatus</i>	14,602	MF975702.1
		<i>Hottentotta tamulus</i>	14,990	PP755025
		<i>Leiurus quinquestriatus</i>	15,083	PP818816
		<i>Lychas mucronatus</i>	14,534	PP874230
		<i>Mesobuthus gibbosus</i>	15,983	AJ716204.2
		<i>Mesobuthus martensii</i>	15,034	DQ340065.1
		<i>Mesobuthus martensii</i>	14,840	MN597087.1
		<i>Teruelius flavopiceus</i>	14,504	PP874229
		<i>Tityus serrulatus</i>	14,460	KR024030.1
	Chactidae	<i>Uroctonus mordax</i>	14,840	EU523756.1
	Scorpionidae	<i>Heterometrus longimanus</i>	14,655	KR190462.1
Araneae	Scorpipidae	<i>Scorpiops tibetanus</i>	14,825	MT903349.1
	Vaejovidae	<i>Vaejovis smithi</i>	14,492	KX520650.1
	Salticidae	<i>Asemonea sichuanensis</i>	15,419	MN651970.1

brates, as the sequences of more and more mitogenomes have been obtained, it has become clear that there exists a degree of variability in the arrangement of mitochondrial genes, which is specific to individual groups and relatively consistent over time (Boore 1999; Cameron 2014). Various mechanisms are commonly suggested to account for these rearrangements, including inversion, transposition, intramolecular recombination, tandem duplication, and deletion. Specifically, tandem duplication and multiple deletions have been proposed as potential mechanisms responsible for the majority of observed rearrangements (Moritz and Brown 1987; Pereira 2000; Moreno-Carmona et al. 2023).

In recent decades, research focus has shifted to gene rearrangements as a valuable phylogenetic characteristic (Lavrov and Lang 2005; Dowton et al. 2009; Feng et al. 2020; Sterling-Montealegre and Prada 2024). At the same time, advances in the field of molecular biology and the development of next-generation sequencing techniques have hastened the process of sequencing complete mitogenomes for numerous species (Feng et al. 2020). As of February 2024, over 30,000 Arthropoda complete mitogenomes were available in the NCBI GenBank (<https://www.ncbi.nlm.nih.gov/>). Among them, taxa such as Arachnida were represented by relatively few complete mitogenomes, at less than 2,000. The order Scorpiones, known for its species richness and morphological diversity (Dávila et al. 2005; Howard et al. 2019), is one of the groups underrepresented with only eleven mitogenomes available in the NCBI (GenBank), most of them belonging to the family Buthidae (Dávila et al. 2005; Choi et al. 2007; Masta and Boore 2008; Martins et al. 2016; Yamashita et al. 2017; Zhang et al. 2020; Zheng and Xiang 2021). In the mitogenomes of scorpions belonging to this family, some rearrangements have been documented, notably the absence of *tRNA-Asp* (Gantenbein et al. 2005; Moreno-Carmona et al. 2023). It is noteworthy that the mitogenome structure of the majority of scorpions remains

uncharacterized (Pons et al. 2019). While the utilization of individual mitochondrial genes (such as *COX1*) has become widespread in phylogenetic and phylogeographic studies (Froufe et al. 2008; Sousa et al. 2012; Li et al. 2022; Intirach et al. 2023), complete mitogenomes offer the opportunity to extract a richness of detailed information at a high resolution and in significant quantities (Sullivan et al. 2017; Sterling-Montealegre and Prada 2024).

In this study, we sequenced the complete mitogenomes of five scorpion species (*Androctonus amoreuxi* Audouin, 1826, *Hottentotta tamulus* Fabricius, 1798, *Leiurus quinquestriatus* Ehrenberg, 1828, *Lychas mucronatus* Fabricius, 1798, and *Teruelius flavopiceus* Kraepelin, 1900) for the first time and analyzed them in comparison with other Scorpiones complete mitogenomes published in the NCBI (as of February 2024). We determined the nucleotide composition, codon usage, gene order, and phylogenetic relationship of a total of 16 complete mitogenomes, with the aim of discovering the patterns of gene rearrangements within Scorpiones, particularly within the family Buthidae, as well as supplementing the mitochondrial data of this order.

## 2. Materials and Methods

### 2.1. Sample collection and DNA extraction

Samples used in this study were collected from insect markets in Linyi (Shandong, China) and Jieyang (Guangdong, China). The collected specimens were firstly morphologically characterized based on the images and morphological features on GBIF (<https://www.gbif.org/>), then identified through molecular identification according to the *COX1* published on NCBI. The collection of spec-

imens was reviewed and approved by Nanjing Forestry University, which conformed to the relevant Chinese laws. The animals were raised at the Zoology Laboratory of Nanjing Forestry University. Total DNA was extracted from muscular tissue using a FastPure Cell/Tissue DNA Isolation Mini Kit (Vazyme™, Nanjing, China). DNA was stored at  $-20^{\circ}\text{C}$  for follow-up.

## 2.2. Next-generation sequencing

Library construction and sequencing were conducted by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) utilizing the NovaSeq X Plus platform (Illumina, USA) for 150 bp paired-end reads.

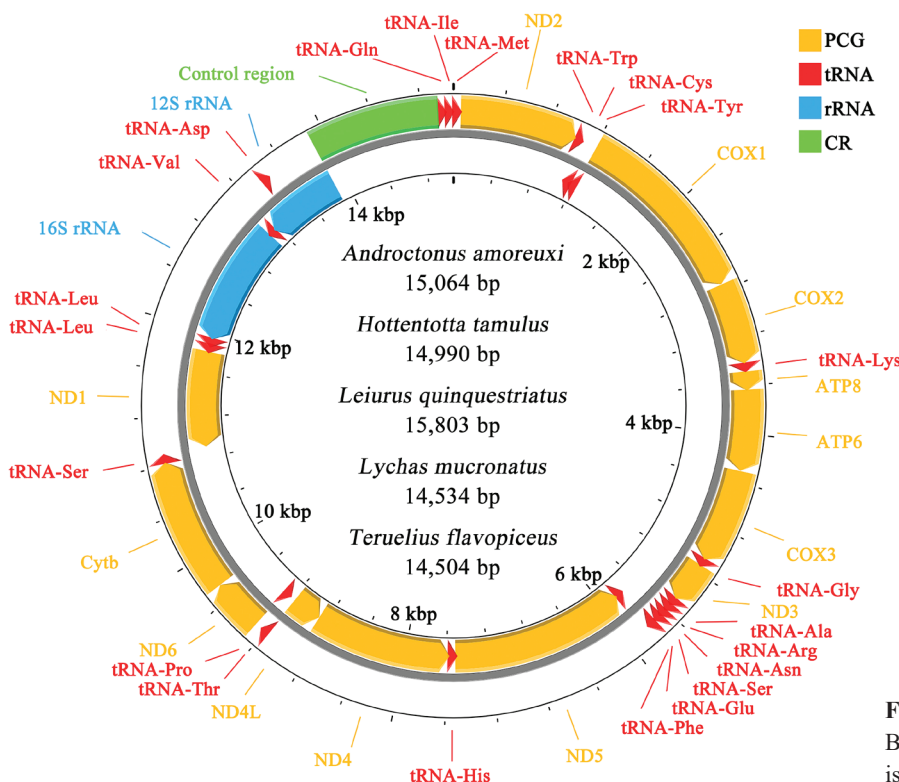
In order to produce high-quality data, sequences of low quality were eliminated. Quality filtering included a sliding window approach for quality assessment. The window size was set to 9 bp, with a step size of 1 bp. The window was moved forward by one base each time, and the average Q-value for the 9 bases within the window was calculated. If the average Q-value of the window was less than 2, only the second-to-last base and all preceding bases within that window were retained. The clean reads were taken for the assembly of the complete mitogenomes, using the Geneious Prime 2023 software and *Buthus occitanus* (Amoreux, 1789) (EU523755.1) as a reference template (Masta and Boore 2008). To construct the circular mitogenomes, both ends of the final assembly were manually examined for any potential overlap. The assembly was conducted under the medium sensitivity/speed setting. Consensus sequences were derived with a 50% base call threshold, leading to the acquisition of the full mitochondrial genomes.

## 2.3. Annotation and sequence analysis

Conservative domains of the mitogenomes were identified using BLAST CD-Search (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) and the MITOS server (<http://mitos.bioinf.uni-leipzig.de/index.py>) (Marchler and Bryant 2004; Bernt et al. 2013). Some tRNAs not detected by MITOS, such as *tRNA-Asp*, were determined using BioEdit software according to tRNAs of other Buthidae species. Gene maps of the mitogenomes were generated using the CG View server (<http://cgview.ca>) (Grant et al. 2023). Nucleotide bias was measured using the formulas “AT-skew =  $(A-T) / (A+T)$ ” and “GC-skew =  $(G-C) / (G+C)$ ” (Perna and Kocher 1995). The analyses of relative synonymous codon usage (RSCU), along with non-synonymous (Ka) and synonymous substitutions (Ks), were conducted using MEGA X software (Kumar et al. 2018). Images of RSCU were output by PhyloSuite v1.2.3 (Zhang et al. 2020), and tRNA genes were identified using the MITOS server.

## 2.4. Phylogenetic analysis

Sixteen species from five families of Scorpiones, as well as *Asemonea sichuanensis* (Song & Chai, 1992) from Araneae-Salticidae as an outgroup taxon, were used for the phylogenetic analyses (Table 1). Sequence alignment and subsequent model prediction was performed using MAFFT v7.505 (Kato and Standley 2013) and ModelFinder v2.2.0 (Kalyaanamoorthy et al. 2017), respectively. Phylogenetic analyses were conducted for each dataset using the Bayesian inference (BI) and maximum



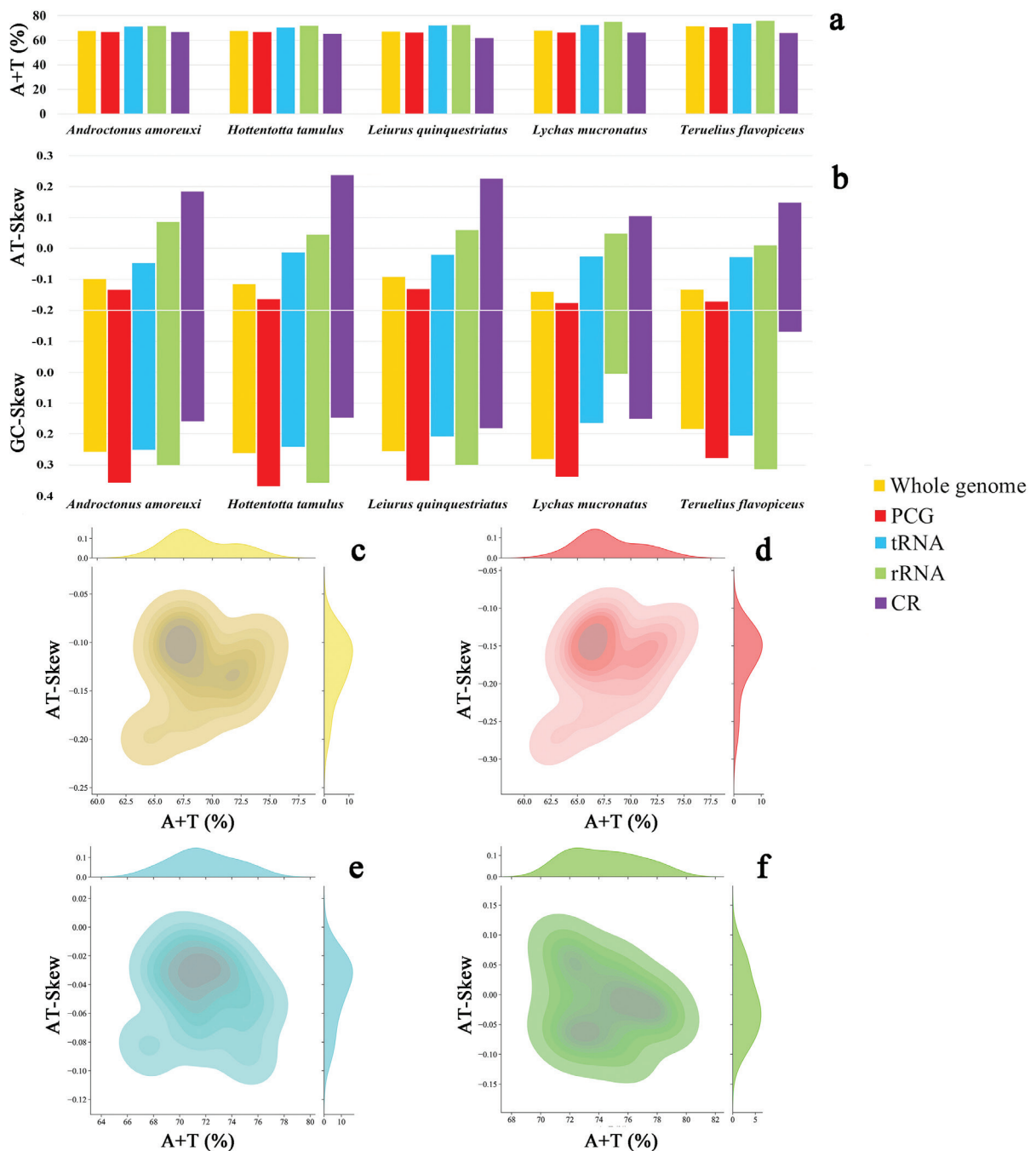
**Figure 1.** Mitochondrial genome of five Buthidae species. Note that *H. tamulus* is the only species lacking *tRNA-Asp*.

likelihood (ML) methods, both available in the Phylo-Suite v1.2.3 (Zhang et al. 2020). The best-fit model of BI according to BIC was determined as GTR+F+I+G4, while for ML, it was GTR+F+I+R3. The BI tree was reconstructed using MrBayes v3.2.7a (Ronquist et al. 2012). Markov chains were run for a million generations and were sampled every 100 generations. The consensus trees based on majority rule were assessed by combining the outcomes of duplicated analyses while discarding the first 25% of generations. The ML tree was reconstructed using IQ-TREE v2.2.0 with 5000 bootstrap replicates (Nguyen et al. 2015). The phylogenetic tree was visualized and edited using the iTOL server (<https://itol.embl.de>) (Letunic and Bork 2021).

### 3. Results

#### 3.1. Mitochondrial genome organization

The five scorpions' complete mitogenomes were typically circular, double-stranded molecules, with sizes of 15,064 bp, 14,990 bp, 15,083 bp, 14,534 bp, and 14,504 bp (Fig. 1). Among them, *T. flavopiceus* had the smallest mitogenome, while *L. quinquestriatus* had the largest. All mitogenomes contained 13 PCGs, 22 tRNAs, two rRNAs, and one CR (Table S1), except for *H. tamulus*, with 21 tRNAs and lacking *tRNA-Asp*. A total of



**Figure 2.** Nucleotide composition of five Buthidae mitogenomes: A+T content (a) and Skewness (b); AT-skew vs A+T content in the Scorpiones mitogenomes: Whole genome (c), PCG (d), tRNA (e), and rRNAs (f).



14 genes were encoded on the minor strand, including 4 PCGs, 8 tRNAs and 2 rRNAs, while the remaining genes were located on the major strand (Table S1).

The nucleotide composition analysis indicated that five mitogenomes displayed a preference for Adenine (A) and Thymine (T), as illustrated in Fig. 2a. All mitogenomes exhibited an A+T content exceeding 67%. This AT bias (A+T > G+C) was also observed in PCGs, RNAs and CRs, with rRNAs showing the highest A+T content. The findings of skewness analysis revealed that the AT skews of five mitogenomes exhibited all negative values, while the GC skews were positive (Fig. 2b). To determine the nucleotide composition on our sampled representatives of the order Scorpiones (mainly the family Buthidae), the A+T content and AT skew were computed for 16 mitogenomes, encompassing five families: Buthidae, Chactidae, Scorpionidae, Scorpipidae, and Vaejoidea (Fig. 2c, d, e, f).

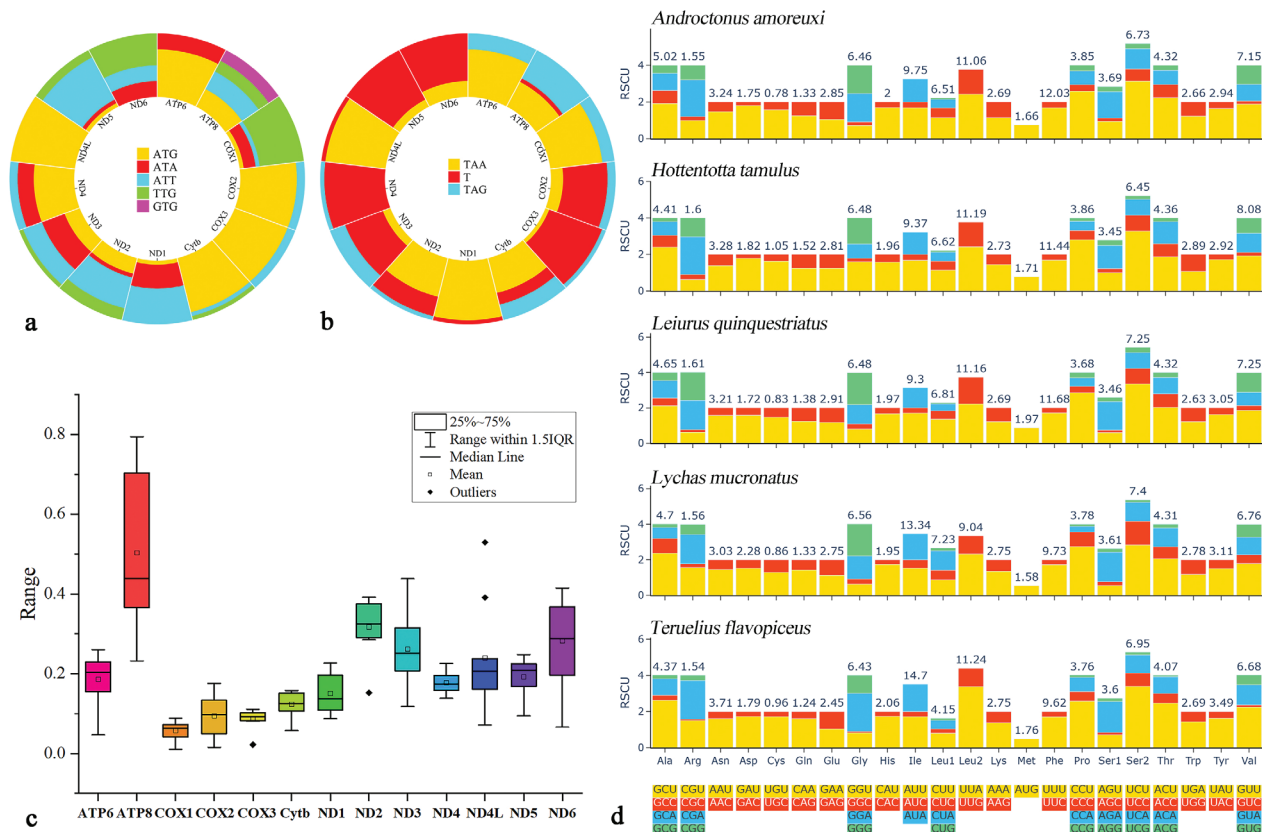
The studied mitochondrial genomes were compact, with intergenic spaces not exceeding 24 bp and gene overlaps not longer than 52 bp (Table S1). Multiple overlaps between adjacent genes were detected: nine gene overlaps were observed in *A. amoreuxi*, six in *H. tamulus*, ten in *L. quinquestriatus* and *L. mucronatus*, and seven in *T. flavopiceus*. The largest overlap was located between *12S rRNA* and *tRNA-Asp*.

### 3.2. Protein-coding genes and codon usage

The total sizes of the PCGs were 10,854 bp, 10,854 bp, 10,867 bp, 10,818 bp, and 10,807 bp, respectively, accounting for 72.05% (*L. quinquestriatus*) to 74.51% (*T. flavopiceus*) of their whole mitogenomes. All PCGs were encoded on the major strand, except for *ND5*, *ND4*, *ND4L*, and *ND1*, which were encoded on the minor strand (Table S1). Among the 13 PCGs presented in these five mitogenomes, *ATP8* exhibited the smallest size, while *ND5* displayed the largest.

The majority of PCGs in the five mitogenomes started with the ATG codon, while the rest used alternative initiation codons such as those found in other animal mitochondrial genomes like ATA, ATT, TTG, and GTG. The termination codon varied across these PCGs, with codons of TAA, TAG, and T. The frequency of the termination codon TAA was consistently higher than that of the other two termination codons, whereas the occurrence of the termination codon TAG was the lowest. Next, an analysis of the initiation and termination codon usage in 16 mitogenomes was conducted (Fig. 3a, b).

This study examined the evolutionary trajectory of PCGs through the analysis of Ka/Ks ratios (Fig. 3c). These ratios for all PCGs were found to be below 1. Sub-



**Figure 3.** Initiation codon (a) and termination codon (b) usage for the mitochondrial genome protein-coding genes of Scorpiones mitogenomes; Ka/Ks values for the 13 PCGs of five Buthidae mitogenomes in this study (c); Relative synonymous codon usage of five Buthidae mitogenomes in this study (d), the termination codon is not included.

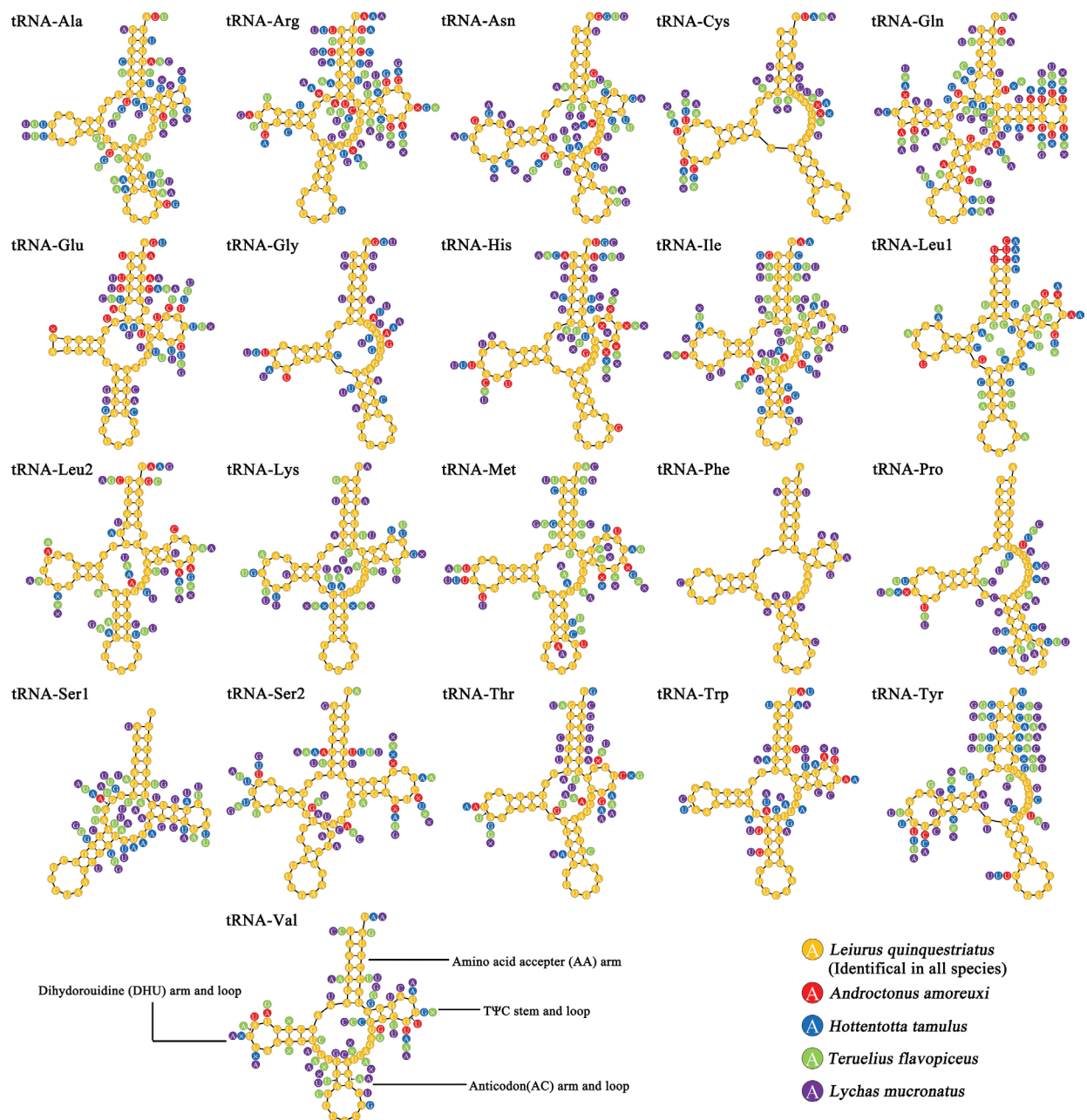
sequently, an analysis of RSCU was carried out to explore the codon usage patterns across the five mitogenomes (Fig. 3d). The RSCU values for the five mitogenomes displayed a notable level of similarity. Moreover, the RSCU analysis indicated a preference for A/T nucleotides at the third codon position (excluding Met), aligning with the observed biased usage of A+T nucleotides, as reflected in the PCGs.

### 3.3. Transfer RNA, ribosomal RNA genes and control regions

Twenty-two tRNAs of *A. amoreuxi*, *L. mucronatus*, *L. quinquestriatus*, and *T. flavopiceus*, and twenty-one tRNAs of *H. tamulus* mitogenomes were scattered discontinuously

over the complete mitogenomes (Table S1). *H. tamulus* lacked *tRNA-Asp*. The tRNA regions of these five mitogenomes were 1,313, 1,285, 1,309, 1,298, and 1,305 bp, accounting for 8.72%, 8.57%, 8.68%, 8.93%, and 9.00%, respectively, of the whole mitogenomes. These five mitogenomes have 22 (or 21) typical tRNA genes, with eight transcribed from the minor strand. The secondary structures of these tRNAs are shown in Fig. 4. Excluding the classic AU and CG pairs, a number of mismatched base pairs were found in different stems, such as AC mismatches, CU mismatches, and AG mismatches were found in these tRNAs.

In the five mitogenomes, two rRNA genes (*12S rRNA* and *16S rRNA*) were transcribed from the minor strand (Table S1). The larger rRNA (*16S rRNA*) was found between *tRNA-Leu1* and *tRNA-Val*, while the smaller



**Figure 4.** Secondary structure of tRNA genes from the five Buthidae mitogenomes, visually illustrating the variations observed among the tRNAs across these mitogenomes.

rRNA (*12S rRNA*) was located between *tRNA-Val* and *tRNA-Gln*. The sizes of *12S rRNA* ranged from 718 to 728 bp, and those *16S rRNA* from 1,151 to 1,160 bp in the mitogenomes.

In the five mitogenomes, CRs were found between the genes *12S rRNA* and *tRNA-Gln* (Table S1). Their sizes ranged from 513 bp (*T. flavopiceus*) to 1,078 bp (*L. quinquestriatus*), accounting for 3.54% to 7.15% of the whole mitogenomes. This accounted for a significant variation, which in turn influenced the overall size of the complete mitogenome. The AT skew of CRs exhibited consistently higher values than PCGs and rRNAs, indicating a higher occurrence of A than T (Fig. 2a).

### 3.4. Gene rearrangement

The structures of the five mitogenomes sequenced in this study differed in tRNAs. As mentioned above, unlike the “*tRNA-Val*, *tRNA-Asp*, *tRNA-Gln*” genetic arrangement of the other four scorpions, *H. tamulus* lacked *tRNA-Asp* to form “*tRNA-Val*, *tRNA-Gln*” (Table S1).

We also summarized and organized the gene arrangement of the five mitogenomes along with other published Scorpiones mitogenomes (Fig. 5). Six Buthidae species lacked *tRNA-Asp*: *Mesobuthus martensii* (Karsch, 1879), *B. occitanus*, *Centruroides vittatus* (Say, 1821), *Centruroides limpidus* (Karsch, 1879), *H. tamulus*, and *Tityus serrulatus* (Lutz & Mello, 1922). The tRNAs adjacent to *tRNA-Asp* in the remaining five Buthidae species were *tRNA-Val* and *tRNA-Gln*, whereas the *tRNA-Asp* of scorpions from the other four families (Chactidae, Scorpionidae, Scorpionidae, and Vaejovidae) was located between *tRNA-Lys* and *ATP8*. The *tRNA-Asp* sizes of scorpions from Buthidae were 50–52 bp, generally smaller than those from the other three families (58–62 bp). The gene order of *tRNA-Gln* and *tRNA-Ile* in mitogenomes of Scor-

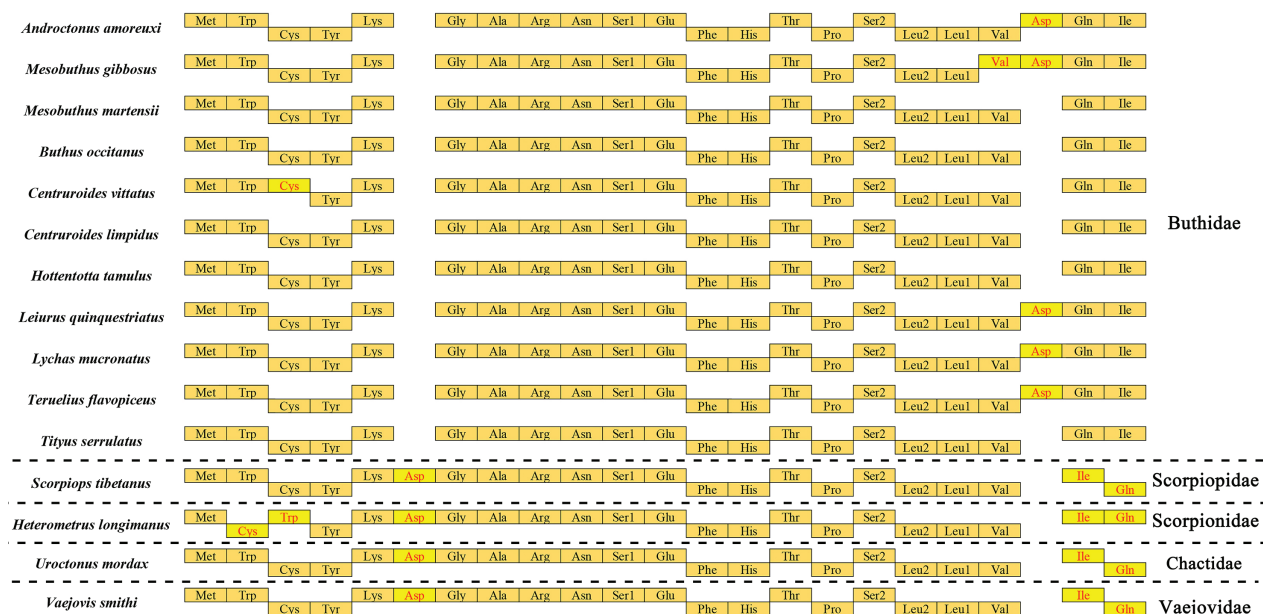
pionidae, Scorpionidae and Vaejovidae was opposite to that of Buthidae. In addition, a small number of gene rearrangement events of *tRNA-Cys* and *tRNA-Val* had occurred.

### 3.5. Phylogenetic analyses

A total of 16 mitogenomes from five families of the order Scorpiones were included in the phylogenetic analyses (Table 1). Additionally, one species from the order Araneae (*A. sichuanensis*) was selected as the outgroup to establish the phylogenetic tree. The BI and ML trees shared an identical topological structure, with well-supported values for most clades (Fig. 6). The five Buthidae mitogenomes analyzed in this study were well clustered together with other mitogenomes of the same family.

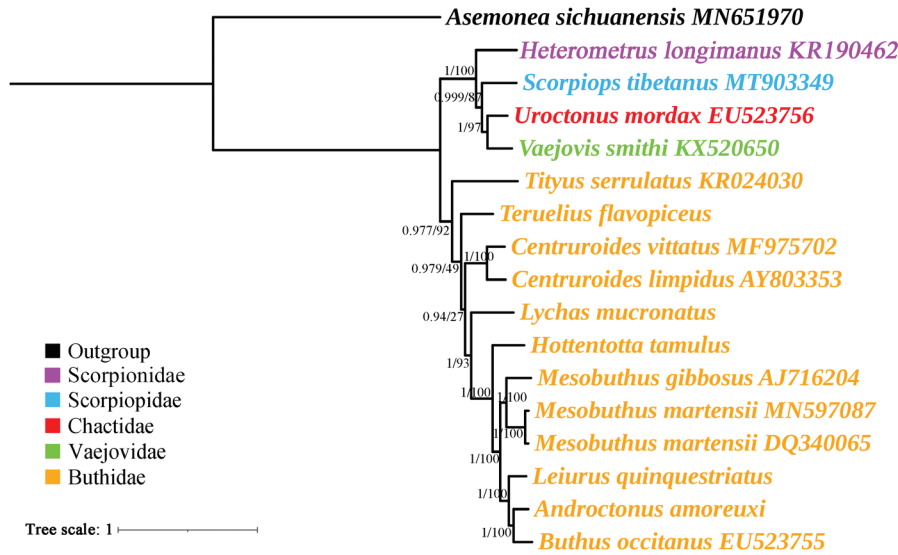
## 4. Discussion

The mitogenomes of the five studied scorpions contained 13 PCGs, 22 tRNAs (or 21 tRNAs), two rRNAs, and one noncoding CR, consistent with findings for other species in the family Buthidae (Gantenbein et al. 2005; Masta and Boore 2008; Moreno-Carmona et al. 2023). For nucleotide composition, five mitogenomes had an AT bias ( $A+T > G+C$ ), which was in agreement with previous studies (Gantenbein et al. 2005; Masta and Boore 2008; Moreno-Carmona et al. 2023). The nucleotide composition of the 16 Scorpiones mitogenomes was found to be relatively consistent, with the A+T content consistently surpassing the G+C content across the whole genome, PCGs, tRNAs, and rRNAs. The AT skews predominantly displayed negative values, suggesting a generally higher



**Figure 5.** tRNAs rearrangement of Scorpiones mitogenomes, including Buthidae, Chactidae, Scorpionidae, Scorpionidae, and Vaejovidae. The light-yellow boxes indicate the events of gene rearrangement.





**Figure 6.** Phylogenetic trees of Scorpiones species and an outgroup taxon (*Araneae: Salticidae: Asemonea sichuanensis*) based on 13 PCGs using the BI and ML methods. Numbers at nodes are statistical support values for BI (posterior probabilities) / ML (bootstrap values).

frequency of occurrence of T compared to A. Notably, the ratio of AT skew to AT content was similar for both the whole genome and PCGs (Fig. 2c, d).

Among the studied Scorpiones species, there was a tendency towards a conservative approach in the selection of initiation codons, predominantly favoring ATG (Fig. 3a, b) (Dávila et al. 2005; Choi et al. 2007; Masta and Boore 2008; Martins et al. 2016; Yamashita et al. 2017; Zhang et al. 2020; Zheng and Xiang 2021). However, exceptions were noted in certain PCGs, such as *COX1* and *ND5*, which predominantly started with TTG and ATT. These alternative initiation codons have been found in other animal mitochondrial genes, including scorpions (Dávila et al. 2005; Silva et al. 2016). All of the considered Scorpiones species shared the termination codons TAA, TAG and T. Specifically, *COX2*, *COX3*, *ND3*, *ND4*, *ND5*, and *ND6* predominantly utilized TAA as the termination codon, while *ATP6*, *COX1*, *Cytb*, *ND1*, *ND2*, and *ND4L* primarily employed T for this purpose. The Ka/Ks ratios of all PCGs were lower than 1 (Fig. 3c), suggesting that purifying selection might play a predominant role in shaping the evolutionary patterns of PCGs. This means that, in most cases, selection eliminates the deleterious mutation, and the protein remains unchanged (Hurst et al. 2002). *COX1* had the lowest average Ka/Ks value, suggesting that it had been under drastic selection pressure and evolved slowly (Hassanin et al. 2005). While *ATP8* had the highest average Ka/Ks value, indicating a high non-synonymous replacement rate of protein-coding sequence, which corresponded to the diverse initiation and termination codons of *ATP8* (Fig. 3a, b, c).

Certain tRNAs could not be accurately predicted in terms of their secondary structures. This phenomenon of tRNA structures has also been reported by a previous study (Yuan et al. 2015). The structure of RNA is involved in various cellular processes, such as transcription, translation and RNA processing (Mustoe et al. 2014). A study has demonstrated that investigating the structure of tRNA poses difficulties due to the extensive modifications and complex framework (Yamagami et al. 2022). The tRNAs found in the five mitogenomes exhibited a higher level of

conservation in the AC arm compared to the other three arms, showing less variation (Fig. 4). The *tRNA-Ser1* lacked the entire DHU arm, as reported previously (Mustoe et al. 2015). The tRNA with the greatest difference was *tRNA-Gln*, which has also been frequently reported to have undergone gene rearrangements in scorpions (Martins et al. 2016; Moreno-Carmona et al. 2023). Notably, among the five examined scorpion species, there was a greater dissimilarity in the tRNAs of *L. mucronatus* and *T. flavopiceus* compared to the other three species. This discrepancy may potentially be explained by the fact that these two species primarily inhabit forest environments, whereas the other three species have adapted to desert habitats (Zhao et al. 2010; El-Aziz et al. 2019; Suranse et al. 2019; Sadine et al. 2023). However, further research would be necessary to confirm this observation.

By obtaining numerous mitogenome sequences, it has been established that while the gene arrangement remains consistent across many invertebrates, rearrangements in a small number of tRNAs are also frequently observed (Cameron 2014; Yoshizawa et al. 2018). Gene rearrangements in our sample representatives of the order Scorpiones (mainly Buthidae) occurred mainly in tRNAs (Fig. 5), more specifically, in deletions of *tRNA-Asp* and translocations of *tRNA-Gln* and *tRNA-Ile*, consistent with previous reports (Gantenbein et al. 2005; Martins et al. 2016; Moreno-Carmona et al. 2023). The deletion of *tRNA-Asp* occurred mainly in the family Buthidae, while the translocations of *tRNA-Gln* and *tRNA-Ile* happened mainly in the families Chactidae, Scorpionidae, Scorpipidae, and Vaejovidae. Among the mitogenomes of the family Buthidae initially published in GenBank (as of February 2024), *Mesobuthus gibbosus* (Brullé, 1832) (AJ716204.2) is the sole mitogenome featuring *tRNA-Asp* (Gantenbein et al. 2005), which is situated within the *12S rRNA*. In this study, the *tRNA-Asp* present in the four mitogenomes was recognized by referencing *M. gibbosus* and was also similarly positioned within the *12S rRNA*.

The results of our phylogenetic analyses based on the complete mitogenomes obtained in this study and published complete Scorpiones mitogenomes were con-



sistent with the classification of the families Buthidae. Furthermore, there are still a large number of Scorpiones species whose complete mitogenomes have not yet been published, and our knowledge on the structure of Scorpiones mitogenomes, especially the pattern and underlying mechanisms of gene rearrangements, is far from comprehensive. Therefore, it is necessary to obtain mitogenome data on further species in this group. Due to the fact that the samples in this study did not originate from their native habitats, but were acquired from commercial sources, we must acknowledge that the presence of these limitations may introduce some bias or uncertainty into our research results. Consequently, in future studies, we need to continue exploring more reliable and accurate sources of samples and identification methods in order to further enhance the accuracy and reliability of related data.

## 5. Conclusion

This study presents the structures and sequencing results of five mitogenomes in the family Buthidae, which are typical circular DNA molecules with sizes ranging from 14,504 to 15,083 bp. Nucleotide composition analysis revealed a bias towards A and T in the sequences. The Ka/Ks ratios of 13 PCGs were found to be below 1, indicating the presence of purifying selection within this family. Therefore, our findings suggest that mitogenome rearrangements in scorpions primarily occur in tRNAs. Specifically, *tRNA-Asp*, *tRNA-Gln* and *tRNA-Ile* were identified as key sites for rearrangements in our sample representatives of the order Scorpiones (mainly Buthidae), and phylogenetic analyses based on PCGs supported the taxonomic relationships within this order. These findings offer valuable insights into the gene arrangement patterns of scorpion mitogenomes and establish a sound basis for future investigations concerning the Buthidae family.

## 6. Declarations

**Authors' contributions.** LHY conceived the study. XW acquired the fund. XW and LZY conducted the sampling. XW, ZGJ, XTJ and WJC conducted the experiments. XW carried out the bioinformatics analysis. XW drafted the manuscript. LHY and HK reviewed and revised the manuscript. All authors approved the final manuscript.

**Funding.** This study was supported by the Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX24\_1382).

**Data availability statement.** DNA sequences: GenBank accession number PP778689 for *Androctonus amoreuxi*, PP755025 for *Hottentotta tamulus*, PP818816 for *Leiurus quinquestriatus*, PP874230 for *Lychas mucronatus*, and PP874229 for *Teruelius flavopiceus*.

**Conflicts of interest.** The authors declare no conflict of interest.

## 7. Acknowledgements

We thank Xinyi Wang for her support of this study and the anonymous reviewers for their work.

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## Supplementary Material 1

### Table S1

**Authors:** Xu W, Zhang GJ, Xu TJ, He K, Wang JC, Liu ZY, Liu HY (2025)

**Data type:** .xlsx

**Explanation notes:** Comprehensive overview of the general features of the mitogenomes of five Buthidae species.

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