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The Complete Mitochondrial Genome of *Coptotettix longtanensis* and its Implications for the Comparative Analysis and Phylogeny of Tetriginae

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ABSTRACT

Mitochondrial genomes are powerful molecular markers for investigating phylogenetic relationships and evolutionary dynamics among closely related taxa. In this study, we sequenced and analyzed the complete mitochondrial genome of *Coptotettix longtanensis* for the first time. The circular mitogenome is 16,861 bp in length and encodes the typical set of 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs), as well as a non-coding A+T-rich control region. The overall A+T content is 74.82%, exhibiting a strong bias consistent with other members of Tetriginae. The gene arrangement is identical to the ancestral insect pattern, except for a *trnK–trnD* translocation. Codon usage analysis revealed a clear preference for A- and T-ending codons, and the Ka/Ks ratios of all 13 PCGs were below 1, indicating strong purifying selection. Phylogenetic relationships among 17 Tetriginae species and two outgroups were reconstructed using Bayesian inference (BI) and maximum likelihood (ML) methods based on concatenated PCGs. Our results showed that the two *Coptotettix* species, *C. longtanensis* and *C. longjiangensis*, formed a distinct and well-supported clade, thus clarifying the phylogenetic position of *C. longtanensis* within Tetriginae. This analysis enhances our understanding of the mitogenome characteristics of Tetriginae grasshoppers and provides a foundation for future phylogenetic research on the family Tetrigidae.

Keywords: Orthoptera, mitogenome, Tetrigidae, pygmy grasshopper, phylogenetic analysis.

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INTRODUCTION

The mitochondrial genome (mitogenome) is typically a double-stranded circular molecule ranging in size from approximately 14,000 to 19,000 base pairs (bp) (Boore, 1999). In insects, the mitogenome generally consists of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and two ribosomal RNA genes (rRNAs) (Cameron, 2014; Clary & Wolstenholme, 1987). In addition, insect mitogenomes usually contain several non-coding regions of variable length, among which the control region (CR) is believed to play a key role in the regulation of mitochondrial DNA replication and transcription (Clary & Wolstenholme, 1987). Owing to its high nucleotide substitution rate, low recombination frequency, compact structure, and lack of introns, the insect mitogenome serves as an efficient molecular marker for studies on species identification, population genetics, molecular evolution, phylogenetics, and phylogeography (Zhang, Wang, Pu, Li, & Song, 2024; Sun et al., 2025; Korkmaz, Aydemir, Temel, Budak, & Başıbüyük, 2017; Liu et al., 2025; Fields et al., 2018). Compared with analyses based on individual mitochondrial genes, complete mitogenomes provide a higher phylogenetic resolution and stronger statistical support (Havird & Santos, 2014). The rapid advancement of next-generation sequencing (NGS) technologies has greatly increased the availability of mitogenomic data across diverse insect orders, thereby offering new opportunities to explore the evolutionary history and phylogenetic relationships within Orthoptera (Li, Wang, Shu, Meng, & Li, 2020; Gaugel, Hawlitschek, Dey, & Husemann, 2023; Bengueraichi, Moussi, Huang, & Husemann, 2024; Zhu et al., 2025).

The pygmy grasshoppers (superfamily Tetrigoidea) represent an important lineage within the order Orthoptera (Deng, 2016). This superfamily includes a single cosmopolitan family, Tetrigidae, comprising more than 300 genera across nine subfamilies with a worldwide distribution (Cigliano, Braun, Eades, & Otte, 2025). Members of this family exhibit distinctive ecological and behavioral adaptations: they primarily feed on decaying organic matter and are typically associated with humid environments such as stream banks, marshes, and mountain brooks (Paranjape & Bacetti, 1987; Kočárek, Holuša, Grucmanová, & Musiolek, 2011). Their limited dispersal ability and reduced flight capacity make them suitable models for investigating environmental adaptation, speciation, and evolutionary radiation (Wei, Xin, & Deng, 2018). However, compared with other orthopteran groups, molecular research on Tetrigidae remains relatively underdeveloped. At present, only 62 mitogenome sequences representing 50 tetrigid species are available in GenBank. Moreover, the distribution of mitogenome data across subfamilies is uneven and fragmentary, underscoring the urgent need for more comprehensive sampling and mitogenomic analyses within this lineage (Li, Ying, Deng, Rong, & Li, 2021; Li, Dou, & Lin, 2025). The genus *Coptotettix*, established by Bolívar in 1887, represents the most diverse group within Tetrigidae and belongs to the subfamily Tetriginae (Zheng, Ou, & Jiang, 2004; Zheng, Lin, & Zhang, 2012). Currently, 79 valid species of *Coptotettix* have been described, distributed mainly across Oceania, Africa, and Asia (Cigliano, Braun, Eades, & Otte, 2025). Despite the high diversity of this genus, molecular data relevant to its phylogenetic and evolutionary relationships remain scarce. To date, only one complete mitogenome of *Coptotettix* has been reported in GenBank.

In this study, we sequenced and annotated the complete mitogenome of *Coptotettix longtanensis* for the first time. We compared its genomic features with those of other published Tetriginae species and reconstructed phylogenetic relationships within the subfamily based on 13 protein-coding genes. Our findings enrich the molecular database of Tetriginae, provide novel insights into the evolutionary relationships of *Coptotettix*, and contribute to a better understanding of the phylogeny of Tetriginae.

MATERIALS AND METHODS

Sample collection

Adult specimens of *Coptotettix longtanensis* were collected from Yanshan, Yunnan Province, China (23°44′45″ N, 104°05′13″ E). The field-collected specimens were initially preserved in 100% ethanol and subsequently stored at −20°C in the laboratory until DNA extraction. Morphological identification was performed based on external characteristics and male genitalia using established taxonomic keys. Voucher specimens were deposited in the College of Life Sciences, Qufu Normal University (China), under accession number HCZ202501.

DNA extraction and sequencing

Total genomic DNA was extracted from thoracic muscle tissues and legs using the Tissue and Blood Genomic DNA Extraction Kit (Qiagen, Germany) following the manufacturer's protocol. DNA concentration and purity were assessed using 1% agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The extracted DNA was stored at −20°C until further use.

High-quality DNA samples were sent to Personal Biotechnology Co., Ltd. (Nanjing, China) for library construction and sequencing. Sequencing libraries were prepared using the TruSeq Nano DNA HT Sample Preparation Kit (Illumina, USA). Paired-end sequencing (PE150) was performed on the Illumina HiSeq 2500 platform with an average insert size of approximately 350 bp, yielding over 4 Gb of clean data per sample.

Mitogenome assembly and annotation

Raw reads generated from the Illumina platform were processed with fastp v0.23.2 to trim adapters, remove low-quality reads and contaminants, and assess read quality using FastQC v0.11.9 (Chen, Zhou, Chen, & Gu, 2018; Brown, Pirrung, & McCue, 2017). Clean reads were *de novo* assembled in Geneious v11.1.5 using the mitogenome of *Coptotettix longjiangensis* (GenBank accession: KY798413) as a reference (Kearse et al., 2012).

Genome annotation was first performed using MitoZ v2.4 and subsequently verified and manually corrected to ensure accuracy (Meng, Li, Yang, & Liu, 2019). The 13 protein-coding genes (PCGs) were compared with *C. longjiangensis* to confirm the start and stop codons. Secondary structures of the 22 tRNA genes were predicted using ARWEN and tRNAscan-SE, and ambiguous regions were manually refined by aligning with homologous sequences from other Orthoptera species (Laslett & Canbäck, 2008; Chan, Lin, Mak, & Lowe, 2021). The positions of the two rRNA genes were identified using MitoZ v2.4 and confirmed with the MITOS web server (Bernt et al., 2013). Intergenic spacers, overlapping regions, and the A+T-rich control region were determined and verified using MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

Sequence analysis

Nucleotide composition, codon usage, and relative synonymous codon usage (RSCU) values were calculated using MEGA X and PhyloSuite v1.2.1 (Zhang et al., 2020). The rates of nonsynonymous (Ka) and synonymous (Ks) substitutions and their ratios (Ka/Ks) for all 13 PCGs were computed using DnaSP v6.0 (Rozas et al., 2017).

Strand compositional asymmetry was calculated with the following formulas: AT-skew = (A − T)/(A + T) and GC-skew = (G − C)/(G + C), where A, T, G, and C represent the frequencies of the four nucleotides. Repeat elements within the A+T-rich region were identified using the Tandem Repeats Finder online tool under default parameters (Benson, 1999).

Phylogenetic analysis

To determine the phylogenetic placement of *C. longtanensis*, mitogenome sequences from 18 additional Tetrigidae species were analyzed. The dataset included 16 published Tetriginae species from GenBank and two outgroup species (*Yunnantettix bannaensis*, Cladonotinae; and *Thoradonta nodulosa*, Scelimeninae). Detailed species information and GenBank accession numbers are provided in Table 1.

Table 1. Information of the mitogenomes analysed in this study.

Subfamily	Genus	Species	Accession number
Tetriginae	*Alulatettix*	*Alulatettix yunnanensis*	JQ272702
	Coptotettix	*Coptotettix longtanensis*	OK540319
		Coptotettix longjiangensis	KY798413
	Ergatettix	*Ergatettix dorsifera*	MN083175
		Ergatettix serrifemora	MN938923
	Euparatettix	*Euparatettix bimaculatus*	MN083177
		Euparatettix variabilis	MN083178
		Euparatettix tridentatus	OR260076
	Exothotettix	*Exothotettix guangxiensis*	OR260077
	Formosatettix	*Formosatettix qinlingensis*	KY798412
	Teredorus	*Teredorus hainanensis*	MZ041207
		Teredorus bashanensis	MZ041208
		Teredorus nigropennis	MN938922
		Teredorus anhuiensis	OP113951
		Teredorus guangxiensis	OR260079
	Tetrix	*Tetrix ruyuanensis*	MN083180
		Tetrix japonica	JQ340002
Scelimeninae	*Thoradonta*	*Thoradonta nodulosa*	MT162547
Cladonotinae	*Yunnantettix*	*Yunnantettix bannaensis*	MN083181

Thirteen PCGs were extracted from all mitogenomes to reconstruct phylogenetic relationships. Each gene was aligned separately using MAFFT v7.205, applying codon-alignment mode for PCGs (Katoh & Standley, 2013). The resulting alignments were concatenated using PhyloSuite. Phylogenetic analyses were performed using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The optimal partitioning scheme and substitution models were determined using PartitionFinder v2.1.1, under the greedy search algorithm, with branch lengths linked across partitions, and model selection based on the Bayesian Information Criterion (BIC). Initial data blocks were defined by gene and codon position (Lanfear, Frandsen, Wright, Senfeld & Calcott, 2017). ML analysis was conducted in IQ-TREE v1.7 with 1,000 bootstrap replicates and automatic model selection (Nguyen, Schmidt, von Haeseler, & Minh, 2015). BI analysis was carried out using MrBayes v3.2.6 with two independent Markov Chain Monte Carlo (MCMC) runs of 1,000,000 generations, sampling every 1,000 generations and discarding the first 25% as burn-in (Ronquist et al., 2012). The final phylogenetic trees were visualized and edited in FigTree v1.4.2 (Rambaut, 2019).

RESULTS AND DISCUSSION

General features of mitogenome

The complete mitogenome (mitogenome) of *Coptotettix longtanensis* was successfully *de novo* assembled and submitted to GenBank under accession number OK540319. The mitogenome is 16,861 bp in length and exhibits the typical circular, double-stranded structure characteristic of insect mitochondrial DNA (Fig. 1). Its size is smaller than that of *Exothotettix guangxiensis* (17,643 bp, GenBank accession: OR260077) but larger than those of other Tetriginae species. The complete mitogenome contains the standard set of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and one non-coding control region (CR, A+T-rich region) (Fig. 1 and Table 2). Among these genes, four PCGs (*nad1*, *nad4*, *nad4L*, and *nad5*), two rRNAs (*rrnS* and *rrnL*), and eight tRNAs (for Cys, Gln, His, Leu1, Phe, Pro, Tyr, and Val) are located on the minority (N) strand. The remaining nine PCGs (*atp6*, *atp8*, *cox1*, *cox2*, *cox3*, *cytb*, *nad2*, *nad3*, and *nad6*) and 14 tRNAs (for Ala, Arg, Asp, Asn, Gly, Glu, Ile, Leu2, Lys, Met, Ser1, Ser2, Thr, and Trp) are located on the majority (J) strand (Table 2). The overall gene order is identical to the inferred ancestral insect arrangement (e.g., *Drosophila yakuba*), except for a *trnK-trnD* translocation (Clary & Wolstenholme, 1985). This rearrangement pattern, also observed in other Acrididea species, can be regarded as a molecular synapomorphy for the group (Li, Shu, Li, Meng, & Li, 2019).

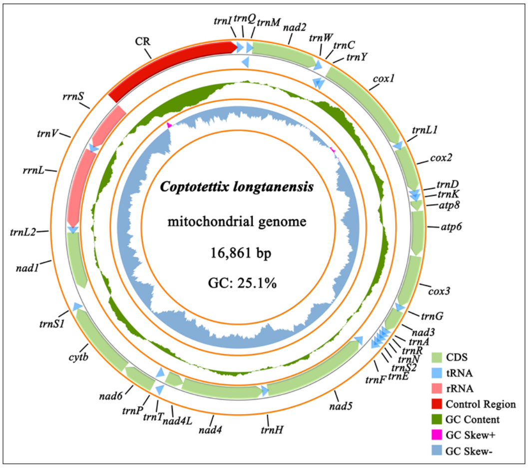

Figure 1. Graphical map of the circular mitogenome of *Coptotettix longtanensis.* Genes transcribed clockwise are shown on the outside, and those transcribed counterclockwise on the inside.

Table 2. Annotation and gene organization of the *Coptotettix longtanensis* mitogenome.

Gene	Strand	From	To	Size (bp)	IN	Anticodon	Start codon	Stop codon
trnI	+	1	65	65	0	GAT		
trnQ	-	77	145	69	11	TTG		
trnM	+	145	213	69	-1	CAT		
nad2	+	214	1,215	1,002	0		GTG	TAA
trnW	+	1,218	1,282	65	2	TCA		
trnC	-	1,275	1,339	65	-8	GCA		
trnY	-	1,342	1,406	65	2	GTA		
cox1	+	1,407	2,937	1,531	0		ATC	T
trnL2	+	2,938	3,001	64	0	TAA		
cox2	+	3,002	3,685	684	0		ATG	TAA
trnD	+	3,684	3,746	63	-2	GTC		
trnK	+	3,747	3,814	68	0	CTT		
atp8	+	3,818	3,976	159	3		ATG	TAA
atp6	+	3,976	4,647	672	-1		ATG	TAA
cox3	+	4,650	5,438	789	2		ATA	TAA
trnG	+	5,438	5,500	63	-1	TCC		
nad3	+	5,501	5,854	354	0		ATA	TAG
trnA	+	5,853	5,916	64	-2	TGC		
trnR	+	5,917	5,978	62	0	TCG		
trnN	+	5,979	6,042	64	0	GTT		
trnS1	+	6,043	6,109	67	0	GCT		
trnE	+	6,110	6,172	63	0	TTC		
trnF	-	6,171	6,233	63	-2	GAA		
nad5	-	6,234	7,950	1,717	0		ATG	T
trnH	-	7,954	8,017	64	3	GTG		
nad4	-	8,017	9,342	1,326	-1		ATG	TAG
nad4L	-	9,336	9,623	288	-7		ATA	TAA
trnT	+	9,629	9,695	67	5	TGT		
trnP	-	9,696	9,759	64	0	TGG		
nad6	+	9,761	10,258	498	1		ATG	TAA
cytb	+	10,258	11,394	1,137	-1		ATG	TAA
trnS2	+	11,408	11,475	68	13	TGA		
nad1	-	11,626	12,564	954	150		ATT	TAA
trnL1	-	12,565	12,627	63	0	TAG		
rrnL	-	12,628	13,929	1,302	0			
trnV	-	13,930	13,997	68	0	TAC		
rrnS	-	13,998	14,778	781	0			
A+T-rich	+	14,779	16,861	2,083	0			

Base composition analysis showed A = 45.32%, T = 29.51%, G = 9.83%, and C = 15.35%, yielding a pronounced A+T bias (74.82%), typical of insect mitogenomes. AT- and GC-skew analyses further indicated A-richness in the complete mitogenome (AT-skew = 0.211), tRNAs (0.038), and CR (0.179). While *rrnL* (GC-skew = 0.274), *rrnS* (0.236), and tRNAs (0.135) were G-rich, suggesting heterogeneous compositional constraints among gene classes (Table 3). Across Tetriginae, mitogenome lengths ranged from 15,086 bp (*Euparatettix tridentatus*) to 17,643 bp (*E. guangxiensis*). The

A+T content varied from 72.18% (*Ergatettix dorsifera*) to 76.82% (*E. guangxiensis*). Genome-wide AT-skew values ranged from 0.175–0.232, whereas GC-skew values were negative (−0.261 to −0.181), indicating A > T and C > G at the whole-genome scale (Table 3). By gene category, tRNAs exhibited positive AT- and GC-skews, while PCGs were negative for both.

Table 3. Base composition and skewness of Tetriginae mitogenomes.

Species	**AT-skew**						**GC-skew**						**A+T(%)**					
	all	**PCGs**	**tRNAs**	***rrnL***	***rrnS***	**CR**	**all**	**PCGs**	**tRNAs**	***rrnL***	***rrnS***	**CR**	**all**	**PCGs**	**tRNAs**	***rrnL***	***rrnS***	**CR**
Alulatettix yunnanensis	0.186	-0.107	0.006	-0.257	-0.266	0.242	-0.207	-0.011	0.154	0.317	0.307	-0.136	75.2	74.6	75.2	78.4	77.3	80.9
Coptotettix longtanensis	0.211	-0.109	0.038	-0.279	-0.244	0.179	-0.219	-0.022	0.135	0.274	0.236	-0.245	74.8	72.1	74.2	78.5	75.6	85.8
Coptotettix longjiangensis	N/A	-0.111	0.028	-0.274	-0.207	N/A	N/A	-0.020	0.104	0.289	0.284	N/A	N/A	72.0	74.7	77.8	76.2	N/A
Ergatettix dorsifera	0.209	-0.112	0.023	-0.271	-0.280	0.251	-0.232	-0.030	0.153	0.295	0.313	-0.11	72.1	70.5	74.3	78.5	76.3	76.1
Ergatettix serrifemora	0.226	-0.112	0.014	-0.266	-0.286	0.389	-0.238	-0.028	0.149	0.306	0.263	-0.321	74.7	73.5	75.2	79.2	77.0	84.3
Euparatettix bimaculatus	0.22	-0.114	0.020	-0.283	-0.319	0.249	-0.261	-0.035	0.159	0.340	0.330	-0.223	72.8	71.8	74.3	77.0	75.8	75.2
Euparatettix tridentatus	0.221	-0.109	0.012	-0.269	-0.248	0.296	-0.261	-0.044	0.147	0.307	0.309	-0.205	73.1	71.8	74.1	76.8	74.2	83.9
Euparatettix variabilis	0.201	-0.115	0.016	-0.268	-0.264	0.272	-0.251	-0.031	0.144	0.331	0.281	-0.302	73.6	72.5	75.2	77.2	74.7	78.4
Exothotettix guangxiensis	0.175	-0.111	0.004	-0.273	-0.238	0.160	-0.181	-0.003	0.151	0.321	0.312	-0.063	76.8	75.1	74.8	79.1	74.9	83.2
Formosatettix qinlingensis	0.188	-0.109	0.010	-0.260	-0.259	0.319	-0.211	-0.005	0.122	0.311	0.308	-0.304	75.6	74.7	75.3	79.3	76.7	80.8
Teredorus anhuiensis	0.230	-0.121	0.066	-0.312	-0.267	0.342	-0.229	-0.033	0.147	0.316	0.291	-0.202	74.0	73.0	73.6	79.0	76.4	74.6
Teredorus bashanensis	N/A	-0.122	0.067	-0.309	-0.306	N/A	N/A	-0.031	0.135	0.321	0.289	N/A	N/A	73.0	73.7	78.8	76.9	N/A
Teredorus guangxiensis	0.232	-0.106	0.013	-0.295	-0.279	0.293	-0.217	-0.041	0.123	0.281	0.284	-0.086	72.7	71.5	73.4	76.5	74.4	81.0
Teredorus hainanensis	N/A	-0.125	0.037	-0.288	-0.263	N/A	N/A	0.002	0.124	0.306	0.273	N/A	N/A	75.6	75.1	79.5	77.5	N/A
Teredorus nigropennis	N/A	-0.142	0.064	-0.295	-0.271	N/A	N/A	-0.018	0.144	0.293	0.284	N/A	N/A	72.4	73.6	77.2	76.3	N/A
Tetrix japonica	0.191	-0.110	0.016	-0.258	-0.266	0.308	-0.214	-0.001	0.142	0.319	0.304	-0.261	75.6	74.7	75.1	79.0	77.0	82.7
Tetrix ruyuanensis	0.189	-0.110	0.019	-0.258	-0.257	0.254	-0.218	-0.002	0.136	0.321	0.297	-0.270	75.5	74.7	75.3	78.9	76.8	79.6

Protein-coding genes

The mitogenome of *C. longtanensis* contains 13 PCGs totaling 11,131 bp, accounting for approximately 66.01% of the entire genome. The concatenated PCGs have an A+T content of 72.1%. The AT-skew was negative (−0.11), and the GC-skew was close to zero (−0.02), indicating more T than A and nearly equal amounts of G and C. Twelve PCGs initiate with typical ATN codons (ATC, ATT, ATA, or ATG), whereas *nad2* starts with GTG (Table 4). Eleven PCGs terminate with complete stop codons (TAG or TAA), while *nad5* and *cox1* end with incomplete T- stop codons. Incomplete codons are commonly observed in insects and other invertebrates, where post-transcriptional polyadenylation restores complete stop signals (Ojala, Montoya, & Attardi, 1981). Across Tetriginae, ATN initiation codons were broadly conserved, though non-canonical starts also occurred (e.g., *nad2* with GTG in *E. bimaculatus* and *E. variabilis*, Table 4). Stop codons were either truncated (T or TA) or complete (TAG or TAA).

The Relative Synonymous Codon Usage (RSCU) profiles showed that UUA (307), AUU (274), and AUA (257) were the most frequently used codons among 3,698 codons (excluding stops), with Leu2 being the most abundant amino acid (Fig. 2 and Table 5). Codons rich in A and T were overrepresented, while C/G-rich codons had RSCU values below 1, reflecting a strong AT bias in codon preference.

Table 4. Start and stop codons of PCGs in Tetriginae mitogenomes.

Species / Gene		***atp6***	***atp8***	***cox1***	***cox2***	***cox3***	***cytb***	***nad1***	***nad2***	***nad3***	***nad4***	***nad4L***	***nad5***	***nad6***
Alulatettix yunnanensis	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATA	ATG	ATA	ATG	ATT	ATG	ATG
	stop	TAA	TAA	TAA	T	TAA	T	TAA	T	TAG	TA	TAA	T	TAA
Coptotettix longjiangensis	**start**	ATG	ATG	ATC	ATG	ATG	ATG	ATT	GTG	ATA	ATG	ATT	ATG	ATG
	stop	TAA	TAA	T	TAA	T	TAA	TAA	TAA	T	TAG	TAA	T	TAA
Coptotettix longtanensis	**start**	ATG	ATG	ACA	ATG	ATA	ATG	ATT	GTG	ATA	ATG	TTG	ATG	ATG
	stop	TAA	TAA	T	TAA	TAA	TAA	TAA	TAA	TAG	TAG	TAA	T	TAA
Ergatettix dorsifera	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATT	ATG	ATC	ATG	ATT	ATG	ATT
	stop	TAA	TAA	T	TAA	T	TAG	TAG	TAA	TAG	TAG	TAA	T	TAA
Ergatettix serrifemora	**start**	ATG	ATG	ATC	ATG	ATG	ATG	ATT	ATT	ATC	ATG	ATT	ATG	TTG
	stop	TAA	TAA	T	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	T	TAA
Euparatettix bimaculatus	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATT	GTG	ATC	ATG	ATT	ATG	ATT
	stop	TAA	TAA	T	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	T	TAA
Euparatettix tridentatus	**start**	ATG	ATG	ATC	ATG	ATG	ATG	ATC	ATG	ATC	ATG	ATT	ATG	ATG
	stop	TAA	TAA	T	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	T	TAA
Euparatettix variabilis	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATT	GTG	ATT	ATG	ATT	ATG	ATG
	stop	TAA	TAA	T	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	T	TAA
Exothotettix guangxiensis	**start**	ATG	ATG	ATC	ATG	ATG	ATG	ATT	ATG	ATT	ATG	ATT	ATG	ATG
	stop	TAA	TAA	T	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	T	TAA
Formosatettix qinlingensis	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATT	ATT	ATA	ATA	ATT	ATG	TTG
	stop	TAA	TAA	TAA	TAA	TAA	TAA	TAA	TAA	TAG	TAG	TAA	T	TAA
Teredorus anhuiensis	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATT	ATA	TTG	ATG	ATT	ATT	TTG
	stop	TAA	TAA	T	TAA	T	TAA	TAA	TAA	TAG	TAG	TAA	T	TAA
Teredorus bashanensis	**start**	ATG	ATG	ATC	ATG	ATG	ATG	ATT	ATG	ATC	ATG	ATT	ATG	ATT
	stop	TAA	TAA	T	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	T	TAA
Teredorus guangxiensis	**start**	ATG	ATG	ATC	ATG	ATG	ATG	ATT	ATG	ATT	ATG	ATT	ATG	ATT
	stop	TAA	TAA	T	TAA	T	TAA	TAA	TAA	TAG	TAG	TAA	T	TAA
Teredorus hainanensis	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATT	ATG	TTG	ATG	ATT	ATG	TTG
	stop	TAA	TAA	T	TAA	T	TAA	TAA	TAA	TAG	TAG	TAA	T	TAA
Teredorus nigropennis	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATA	ATG	ATA	ATG	ATT	ATG	ATG
	stop	TAA	TAA	TAA	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	TAA	TAA
Tetrix japonica	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATT	ATG	ATC	ATG	ATT	ATG	ATG
	stop	TAA	TAA	T	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	T	TAA
Tetrix ruyuanensis	**start**	ATG	ATG	ATC	ATG	ATA	ATG	ATT	ATG	ATC	ATG	ATT	ATG	ATG
	stop	TAA	TAA	T	TAA	T	TAG	TAA	TAA	TAG	TAG	TAA	T	TAA

The Ka/Ks ratio (ω) for 13 PCGs from 17 Tetriginae species is shown in Fig. 3. All genes had Ka/Ks < 1, indicating purifying selection, with values ranging from 0.10 (*cox1*) to 0.55 (*atp8*). This pattern is typical of metazoan mitogenomes and supports the use of *cox1* as a reliable phylogenetic marker within Tetrigidae (Luo, Zhang, & Deng, 2024; Li, Leng, He, Deng, & Guan, 2025).

Table 5. Relative synonymous codon usage (RSCU) of *C. longtanensis* mitogenome.

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	258	1.54	UCU(S)	113	2.58	UAU(Y)	133	1.57	UGU(C)	44	1.69
UUC(F)	78	0.46	UCC(S)	12	0.27	UAC(Y)	36	0.43	UGC(C)	8	0.31
UUA(L)	307	3.56	UCA(S)	108	2.47	UAA(*)	0	0	UGA(W)	81	1.67
UUG(L)	85	0.98	UCG(S)	8	0.18	UAG(*)	0	0	UGG(W)	16	0.33
CUU(L)	49	0.57	CCU(P)	45	1.37	CAU(H)	36	1.14	CGU(R)	19	1.49
CUC(L)	6	0.07	CCC(P)	23	0.7	CAC(H)	27	0.86	CGC(R)	5	0.39
CUA(L)	60	0.69	CCA(P)	57	1.74	CAA(Q)	52	1.63	CGA(R)	22	1.73
CUG(L)	11	0.13	CCG(P)	6	0.18	CAG(Q)	12	0.38	CGG(R)	5	0.39
AUU(I)	274	1.55	ACU(T)	47	0.91	AAU(N)	141	1.52	AGU(S)	28	0.64
AUC(I)	79	0.45	ACC(T)	27	0.52	AAC(N)	45	0.48	AGC(S)	5	0.11
AUA(M)	257	1.66	ACA(T)	129	2.5	AAA(K)	100	1.65	AGA(S)	73	1.67
AUG(M)	52	0.34	ACG(T)	3	0.06	AAG(K)	21	0.35	AGG(S)	3	0.07
GUU(V)	74	1.68	GCU(A)	58	1.54	GAU(D)	59	1.59	GGU(G)	81	1.54
GUC(V)	8	0.18	GCC(A)	29	0.77	GAC(D)	15	0.41	GGC(G)	19	0.36
GUA(V)	78	1.77	GCA(A)	58	1.54	GAA(E)	67	1.65	GGA(G)	63	1.2
GUG(V)	16	0.36	GCG(A)	6	0.16	GAG(E)	14	0.35	GGG(G)	47	0.9

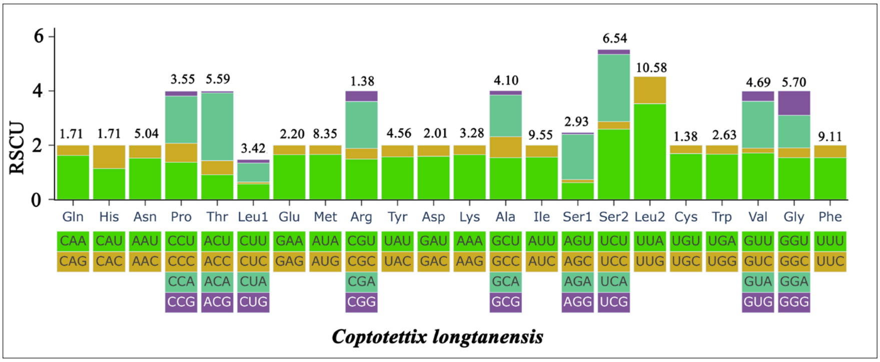

Figure 2. The relative synonymous codon usage (RSCU) in the mitogenome of *Coptotettix longtanensis.*

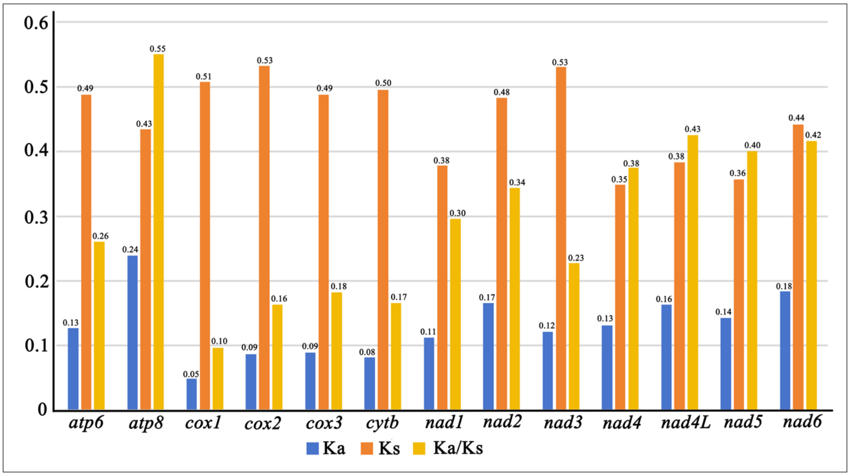

Figure 3. The average Ka/Ks ratio for 13 PCGs among 17 Tetriginae species.

Transfer RNA and ribosomal RNA genes

The mitogenome of *C. longtanensis* contains a standard set of 22 tRNAs: two for Leucine and Serine and one for each remaining amino acid, distributed throughout the genome and ranged in size from 62 bp (*trnR*) to 69 bp (*trnQ*/*trnM*) (Table 2). Anticodons were identical to those of other orthopterans, suggesting high conservation of tRNA identity. Predicted secondary structures are shown in Fig. 4. All tRNAs form typical cloverleaf secondary structures except *trnS1*, which lacks the DHU arm. The missing DHU arm of *trnS1*(AGN) is as a common phenomenon not only in the Tetrigidae also in other insects (Li et al., 2021; Luo et al., 2024; Li et al., 2025). A total of 28 mismatched base pairs were found, among which G-U pair was the most common mismatches, with 20 pairs detected in 15 tRNAs. Meanwhile, two U-U and one A-G mismatched pairs were recognized (Fig. 4). Such non-Watson-Crick pairs are widespread in insects and are generally corrected during post-transcriptional editing, without affecting tRNA functionality (Varani & McClain, 2000).

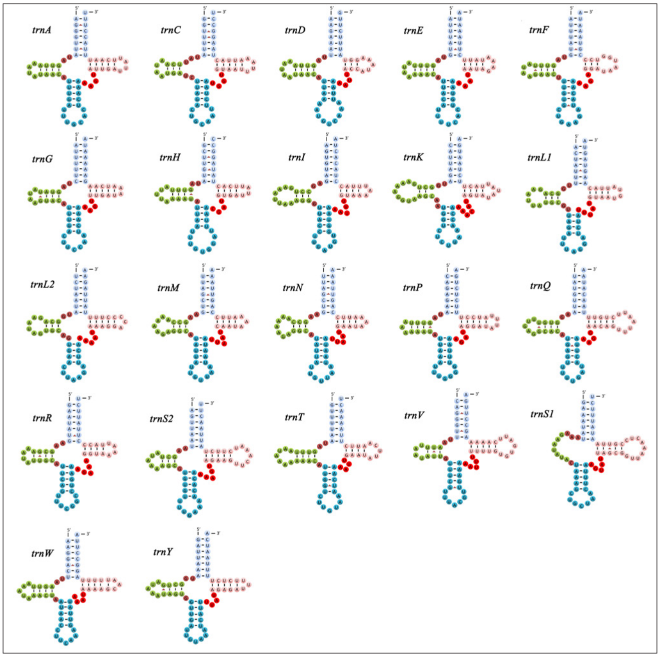

Figure 4. The putative tRNA second structure for the mitogenome of *Coptotettix longtanensis.*

Like other insects, the mitogenome of *C. longtanensis* possesses two rRNAs, *rrnL* and *rrnS*, which encoded the large and small subunit ribosomal RNA, respectively (Li, Dou, & Lin, 2025). The *rrnL* was 1,302 bp with an A + T content of 78.5%, which was located between *trnL1* and *trnV*. The *rrnS* was 781 bp with an A + T content of 75.6%, located downstream of *trnV* and upstream of the A + T-rich region. Both genes showed a negative AT-skew and positive GC-skew, indicating a higher content of T than A and a higher content of G than C. Similar compositional patterns were observed across all 17 Tetriginae species analyzed, suggesting conservation of nucleotide composition within this lineage (Table 3).

Non-coding regions

Differences in mitogenome length among species mainly arise from variation in non-coding regions (Liu et al., 2025; Lv, & Kong, 2018). *C. longtanensis* contains 10 non-coding spacers (excluding the A+T-rich region) totaling 192 bp, with the longest (150 bp) located between *trnS2* and *nad1* (Table 2). Additionally, ten overlapping regions (totaling 26 bp) were identified, the longest (8 bp) between *trnW* and *trnC*.

The A+T-rich control region (CR) is the longest non-coding segment (2,083 bp; A+T = 85.8%) and contains several repetitive sequences of variable length. Two 542 bp repeats occur between positions 671–1,566 bp, separated by a 353 bp spacer, and two 123 bp repeats occur between 1,609–1,900 bp, separated by a 168 bp spacer. Although the CR provides valuable information for intraspecific and population-level analyses, its high A+T content and repetitive nature complicate amplification, assembly, and alignment, limiting its use for deep phylogenetic inference.

Among Tetriginae, AT-skew values in the CR span 0.160–0.342 (highest in *E. guangxiensis*, lowest in *T. anhuiensis*), and GC-skew ranges from −0.302 (*E. variabilis*) to −0.063 (*E. guangxiensis*) (Table 3). The CR of *C. longtanensis* is longer than those of congeners, and such interspecific CR length differences substantially contribute to total mitogenome size variation within Tetriginae.

Phylogenetic analysis

The taxonomic placement of *C. longtanensis* within Tetriginae was determined through phylogenetic analyses conducted using both Bayesian inference (BI) and maximum likelihood (ML) methods. Nucleotide sequences of all 13 PCGs from 19 species including 17 Tetriginae species and two outgroups were combined for phylogenetic analysis. As shown in Fig. 5, the topologies obtained from the two methods were highly congruent, with most nodes showing strong statistical support (BI posterior probabilities = 1.00; ML bootstrap values ≥ 97).

Our results revealed that all genera represented by more than two species were recovered as monophyletic, except for the genus *Ergatettix*, which did not form a single clade. This finding is consistent with the cladistic pattern reported in a recent phylogenetic analysis of Tetrigidae (Luo et al., 2024). The two *Coptotettix* species, *C. longtanensis* and *C. longjiangensis*, clustered together with full statistical support (BI = 1.00, ML = 100), forming a distinct and well-supported lineage that confirms the monophyly of the genus *Coptotettix* and clarifies the phylogenetic position of *C. longtanensis* within Tetriginae (Fig. 5). It is evident from our phylogenetic tree that mitogenome data for Tetriginae species remain extremely limited and unevenly distributed across genera. This data scarcity has, to some extent, constrained the accuracy and resolution of phylogenetic analyses within this subfamily. In future work, we aim to sequence and incorporate additional mitogenomes to provide a more comprehensive understanding of Tetriginae evolutionary relationships.

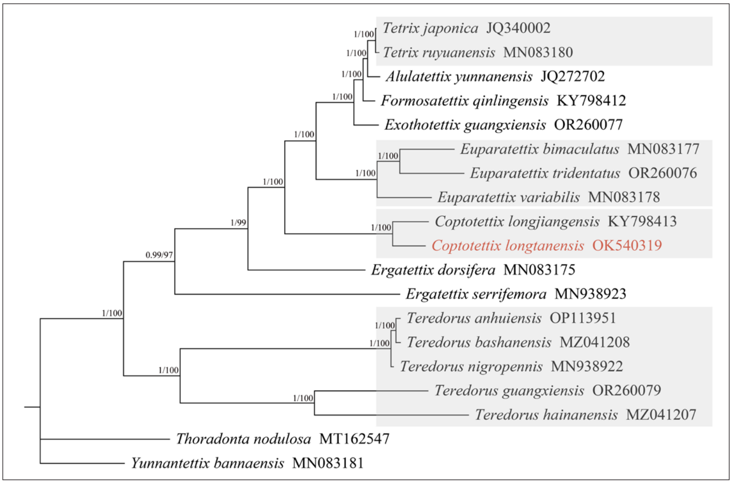

Figure 5. A phylogenetic tree obtained from ML and BI analysis based on the 13 PCGs dataset. Numbers separated by a slash on nodes are posterior probability (PP) and bootstrap value (BV). Gray-shaded clades represent genera that are represented by more than two representative species.

CONCLUSIONS

In this study, we sequenced and analyzed the complete mitogenome of *C. longtanensis*, providing valuable data for elucidating the molecular characteristics and evolutionary relationships within the subfamily Tetriginae. The mitogenome of *C. longtanensis* exhibits the typical circular structure and gene composition found in most orthopteran insects, comprising 37 genes and an A+T-rich control region. Its overall A+T content (74.82%) and gene arrangement, featuring a single trnK–trnD translocation, are consistent with patterns commonly observed in Acrididea species. Codon usage analysis revealed a marked preference for A/T-ending codons. Comparative analysis with other published Tetriginae mitogenomes demonstrated that variations in genome length are mainly attributed to differences in the non-coding control region. The Ka/Ks ratio analysis showed that all 13 protein-coding genes have undergone purifying selection, reflecting strong evolutionary constraints and functional conservation of mitochondrial genes. Phylogenetic reconstruction based on 13 concatenated PCGs revealed that the two *Coptotettix* species, *C. longtanensis* and *C. longjiangensis*, formed a distinct and well-supported clade (BI = 1.00; ML = 100), thereby confirming the monophyly of the genus *Coptotettix* and clarifying the phylogenetic position of *C. longtanensis* within Tetriginae. Overall, our findings expand the mitochondrial genomic resources available for Tetrigidae and provide new insights into the evolutionary patterns of this subfamily. However, the limited and uneven representation of mitogenome data across Tetriginae genera still constrains phylogenetic resolution. Future studies should focus on sequencing additional representative taxa to build a more comprehensive and robust phylogenetic framework, thereby deepening our understanding of the evolutionary diversification of pygmy grasshoppers.

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Data resources

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at [https://www.ncbi.nlm.nih.gov] under the accession no. OK540319. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA776417, SRR16642558, and SAMN22788963, respectively.

Conflicts of interest

The authors have declared that no competing interests exist.

Author contributions

Chao Xue: Conceptualization (lead); software (lead); methodology (equal); writing-original draft (lead). Dan Zhang: Conceptualization (equal); methodology (equal); writing-review & editing (lead). Yujian Li: writing-review & editing (lead). Ran Li: Conceptualization (equal); project administration (lead); supervision (lead); writing-review & editing (lead). Xianfeng Yi: Conceptualization (equal); project administration (lead); supervision (lead); writing-review & editing (lead).

REFERENCES

Bengueraichi, F., Moussi, A., Huang, Y., & Husemann, M. (2024). Characterisation of the complete mitochondrial genomes and phylogenetic analysis of ten Oedipodinae grasshoppers (Orthoptera: Acrididae) from Algeria. *Oriental Insects*, 58(4), 732-765. https://doi.org/10.1080/00305316.2024.2359443

Benson, G. (1999). Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Research*, 27(2), 573-580. https://doi.org/10.1093/nar/27.2.573

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. *Molecular Phylogenetics and Evolution*, 69(2), 313-319. https://doi.org/10.1016/j.ympev.2012.08.023

Boore, J.L. (1999). Animal mitochondrial genomes. *Nucleic Acids Research*, 27(8), 1767-1780. https://doi.org/10.1093/nar/27.8.1767

Brown, J., Pirrung, M., & McCue, L.A. (2017). FQC Dashboard: Integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. *Bioinformatics*, 33(19), 3137-3139. https://doi.org/10.1093/bioinformatics/btx373

Cameron, S.L. (2014). Insect mitochondrial genomics: Implications for evolution and phylogeny. *Annual Review of Entomology*, 59(1), 95-117. https://doi.org/10.1146/annurev-ento-011613-162007

Chan, P.P., Lin, B.Y., Mak, A.J., & Lowe, T.M. (2021). tRNAscan-SE 2.0: Improved detection and functional classification of transfer RNA genes. *Nucleic Acids Research*, 49(16), 9077-9096. https://doi.org/10.1093/nar/gkab688

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34(17), i884-i890. https://doi.org/10.1093/bioinformatics/bty560

Cigliano, M.M., Braun, H., Eades, D.C., & Otte, D. (2025). *Orthoptera Species File* (Version 5.0/5.0). Retrieved March 3, 2025, from http://Orthoptera.SpeciesFile.org

Clary, D.O., & Wolstenholme, D.R. (1985). The ribosomal RNA genes of *Drosophila* mitochondrial DNA. *Nucleic Acids Research*, 13(11), 4029-4045. https://doi.org/10.1093/nar/13.11.4029

Clary, D.O., & Wolstenholme, D.R. (1987). *Drosophila* mitochondrial DNA: Conserved sequences in the A+T-rich region and supporting evidence for a secondary structure model of the small ribosomal RNA. *Journal of Molecular Evolution*, 25(2), 116-125. https://doi.org/10.1007/BF02101753

Deng, W.A. (2016). *Taxonomic study of Tetrigoidea from China* (Doctoral dissertation, Huazhong Agricultural University). (in Chinese)

Fields, P.D., Obbard, D.J., McTaggart, S.J., Galimov, Y., Little, T.J., & Ebert, D. (2018). Mitogenome phylogeographic analysis of a planktonic crustacean. *Molecular Phylogenetics and Evolution*, 129, 138-148. https://doi.org/10.1016/j.ympev.2018.06.028

Gaugel, S.M., Hawlitschek, O., Dey, L.S., & Husemann, M. (2023). Evolution of mitogenomic gene order in Orthoptera. *Insect Molecular Biology*, 32(4), 387-399. https://doi.org/10.1111/imb.12838

Havird, J.C., & Santos, S.R. (2014). Performance of single and concatenated sets of mitochondrial genes at inferring metazoan relationships relative to full mitogenome data. *PLoS One*, 9(1), e84080. https://doi.org/10.1371/journal.pone.0084080

Katoh, K., & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30(4), 772-780. https://doi.org/10.1093/molbev/mst010

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., & Thierer, T. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647-1649. https://doi.org/10.1093/bioinformatics/bts199

Kočárek, P., Holuša, J., Grucmanová, Š., & Musiolek, D. (2011). Biology of *Tetrix bolivari* (Orthoptera: Tetrigidae). *Open Life Sciences*, 6(4), 531-544. https://doi.org/10.2478/s11535-011-0023-y

Korkmaz, E.M., Aydemir, H.B., Temel, B., Budak, M., & Başıbüyük, H.H. (2017). Mitogenome evolution in Cephini (Hymenoptera: Cephidae): Evidence for parallel adaptive evolution. *Biochemical Systematics and Ecology*, 71, 137-146. https://doi.org/10.1016/j.bse.2017.02.004

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., & Calcott, B. (2017). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34(3), 772-773. https://doi.org/10.1093/molbev/msw260

Laslett, D. & Canbäck, B. (2008). ARWEN: A program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics*, 24(2), 172-175. https://doi.org/10.1093/bioinformatics/btm573

Li, R., Shu, X.H., Li, X.D., Meng, L., & Li, B.P. (2019). Comparative mitogenome analysis of three species and monophyletic inference of Catantopinae (Orthoptera: Acridoidea). *Genomics*, 111(6), 1728-1735. https://doi.org/10.1016/j.ygeno.2018.11.027

Li, R., Wang, Y., Shu, X.H., Meng, L., & Li, B.P. (2020). Complete mitochondrial genomes of three *Oxya* grasshoppers (Orthoptera) and their implications for phylogenetic reconstruction. *Genomics*, 112(1), 289-296. https://doi.org/10.1016/j.ygeno.2019.02.008

Li, R., Ying, X.L., Deng, W.A., Rong, W.T., & Li, X.D. (2021). Mitochondrial genomes of eight Scelimeninae species (Orthoptera) and their phylogenetic implications within Tetrigoidea. *PeerJ*, 9, e10523. https://doi.org/10.7717/peerj.10523

Li, X.J., Dou, W.L., & Lin, L.L. (2025). Mitogenomic phylogeny of Tetrigoidea (Insecta, Orthoptera), with a focus on the genus *Zhengitettix*. *PeerJ*, 13, e19521. https://doi.org/10.7717/peerj.19521

Li, Y.M., Leng, S.X., He, J.S., Deng, W.A., & Guan, D.L. (2025). Mitochondrial phylogenomics of pygmy grasshoppers (Orthoptera: Tetrigidae: Metrodorinae): Descriptions of a new genus, two new species, and new synonyms from China. *ZooKeys*, 1236, 249-272. https://doi.org/10.3897/zookeys.1236.145914

Liu, Q.Q., Zhu, P.Z., Xu, S.W., Yang, C.Y., Song, F., Meng, Y.F., Zhou, J.H., Yang, H.L. & Huang, W. D. (2025). Comparative mitogenomes and phylogenetic analyses of Coccinellidae (Coleoptera: Coccinelloidea). *Ecology and Evolution*, 15(3), e71053. https://doi.org/10.1002/ece3.71053

Luo, J.L., Zhang, R.J., & Deng, W.A. (2024). First mitogenomic characterization of *Macromotettixoides* (Orthoptera: Tetrigidae), with the descriptions of two new species. *ZooKeys*, 1195, 95-112. https://doi.org/10.3897/zookeys.1195.112623

Lv, C., Li, Q., & Kong, L. (2018). Comparative analyses of the complete mitochondrial genomes of *Dosinia* clams and their phylogenetic position within Veneridae. *PLoS One*, 13(4), e0196466. https://doi.org/10.1371/journal.pone.0196466

Meng, G., Li, Y., Yang, C., & Liu, S. (2019). MitoZ: A toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Research*, 47(11), e63. https://doi.org/10.1093/nar/gkz173

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. *Molecular Biology and Evolution*, 32(1), 268-274. https://doi.org/10.1093/molbev/msu300

Ojala, D., Montoya, J., & Attardi, G. (1981). tRNA punctuation model of RNA processing in human mitochondria. *Nature*, 290(5806), 470-474. https://doi.org/10.1038/290470a0

Paranjape, S.Y. & Bacetti, B.M. (1987). Etho-ecological characteristics and phylogeny of Tetrigidae. In B. M. Bacetti (Ed.), *Evolutionary biology of orthopteroid insects* (pp. 386-395). Chichester, UK: Ellis Horwood.

Rambaut, A. (2019). *FigTree v1.4.2*. Institute of Evolutionary Biology, University of Edinburgh. Retrieved from http://tree.bio.ed.ac.uk/software/figtree/

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3), 539-542. https://doi.org/10.1093/sysbio/sys029

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 34(12), 3299-3302. https://doi.org/10.1093/molbev/msx248

Sun, S., Li, S., Seim, I., Du, X., Yang, X.W., Liu, K.Q., Wei, Z.F., Shao, C.W., Fan, G.Y., & Liu, X. (2025). Complete mitogenomes reveal high diversity and recent population dynamics in Antarctic krill. *BMC Genomics*, 26(1), 419. https://doi.org/10.1186/s12864-025-11579-w

Varani, G. & McClain, W.H. (2000). The G-U wobble base pair: A fundamental building block of RNA structure crucial to RNA function in diverse biological systems. *EMBO Reports*, 1(1), 18-23. https://doi.org/10.1093/embo-reports/kvd001

Wei, S.Z., Xin, L., & Deng, W.A. (2018). Pygmy grasshoppers of the genus *Paragavialidium* Zheng, 1994 (Orthoptera: Tetrigoidea: Scelimeninae). *Oriental Insects*, 53(4), 449-469. https://doi.org/10.1080/00305316.2018.1503976

Zhang, D., Gao, F., Jakovlić, I., Zou, H., Zhang, J., Li, W.X., & Wang, G.T. (2020). PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, 20(1), 348-355. https://doi.org/10.1111/1755-0998.13096

Zhang, N., Wang, J., Pu, T., Li, C., & Song, Y. (2024). Two new species of Erythroneurini (Hemiptera: Cicadellidae: Typhlocybinae) from southern China based on morphology and complete mitogenomes. *PeerJ*, 12, e16853. https://doi.org/10.7717/peerj.16853

Zheng, Z.M., Lin, L.L., & Zhang, H.L. (2012). A taxonomic study of the genus *Coptotettix* Bolívar, 1887 (Orthoptera: Tetrigidae: Tetriginae) from China with description of a new species. *Journal of Natural History*, 46(41-42), 2549-2561. https://doi.org/10.1080/00222933.2012.708448

Zheng, Z.M., Ou, X.H., & Jiang, G.F. (2004). A taxonomic review of the genus *Coptotettix* Bolívar from China (Orthoptera: Tetrigidae). *Acta Entomologica Sinica*, 47(1), 80-85. (in Chinese)

Zhu, W., Guan, D., Chen, Z., Dey, L.S., Huang, H., Li, X., Fondjo, J.A., Hawlitschek, O., Zhang, Z., Husemann, M., & Xu, S.Q. (2025). Mitogenomics provide insights into the tribe-level systematics and historical phylogeography of band-winged grasshoppers (Orthoptera: Acrididae: Oedipodinae). *Cladistics,* 41(6), 523-544. https://doi.org/10.1111/cla.70006