The complete mitochondrial genomes of four lagriine species (Coleoptera, Tenebrionidae) and phylogenetic relationships within Tenebrionidae

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ABSTRACT

It is common to use whole mitochondrial genomes to analyze phylogenetic relationships among insects. In this study, seven mitogenomes of Tenebrionidae are newly sequenced and annotated. Among them, four species (*Cerogira janthinipennis* (Fairmaire, 1886), *Luprops yunnanus* (Fairmaire, 1887), *Anaedus unidentasus* Wang & Ren, 2007, and *Spinolyprops cribricollis* Schawaller, 2012) represent the subfamily Lagriinae. In this subfamily, the mitogenomes of the tribes Goniaderini (*A. unidentasus*) and Lupropini (*L. yunnanus* and *S. cribricollis*) were first reported; they were found to be 15,328–16,437 bp in length and encode 37 typical mitochondrial genes (13 PCGs, 2 rRNAs, 22 tRNAs, and a single noncoding control region). Most protein-coding genes in these mitogenomes have typical ATN start codons and TAR or an incomplete stop codon T–. In these four lagriine species, F, L2, I, and N are the most frequently used amino acids. In the 13 PCGs, the gene *atp8* (Pi = 0.978) was the most diverse nucleotide, while *cox1* was the most conserved gene with the lowest value (Pi = 0.211). The phylogenetic results suggest that Pimelinae, Lagriinae, Blaptinae, Stenochiinae, and Alleculinae are monophyletic, Diaperinae is paraphyletic, and Tenebrioninae appears polyphyletic. In Lagriinae, the tribe Lupropini appears paraphyletic because *Spinolyprops* is clustered with *Anaedus* in Goniaderini. These mitogenomic data provide important molecular data for the phylogeny of Tenebrionidae.

Subjects Entomology, Genomics, Taxonomy, Zoology
Keywords Darkling beetles, Lagriinae, Mitogenome, Phylogenetic analysis

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INTRODUCTION

The subfamily Lagriinae belongs to the family Tenebrionidae, which includes 11 subfamilies (*Bouchard et al., 2011*; *Nabozhenko & Sadeghi, 2017*; *Kamiński et al., 2021*), 11 tribes, and 273 valid genera that are widely distributed in the world (*Bouchard et al., 2021*). Most of the lagriine adults are herbivorous and mycophagous. Their larvae are forest-floor dwellers commonly found in leaf litter and other accumulations of dead plant matter (*Matthews et al., 2010*).

Although some taxonomists have made contributions to the higher-level classification of Lagriinae based on morphological characteristics (*Doyen & Tschinkel, 1982*; *Doyen,*

Matthews & Lawrence, 1989; *Bouchard et al., 2021*) and molecular phylogeny (*Kergoat et al., 2014*; *Aalbu, Kanda & Smith, 2017*; *Wu et al., 2022*), the problems of classification remain unresolved. For example, the tribe Lupropini is paraphyletic. The genera *Spinolyprops* and *Sphingocorse* are placed in the tribe Lupropini; however, the morphological characteristics of this genus are more suitable for the tribe Goniaderini. Many genera of the tribe Lupropini are similarly situated and further studies are needed to resolve these discrepancies.

Mitogenomes have provided valuable data on phylogeny (*Saccone et al., 1999*; *Cameron, 2014*; *Li et al., 2015*; *Qin et al., 2015*; *Nie et al., 2020*; *Tian, Yuan & Chen, 2020*; *Nie et al., 2021*), evolution strategies (*Krzywinski et al., 2011*; *Motyka et al., 2022*; *Nie et al., 2019*), and species delimitation (*Kim et al., 2021*) over the past few decades. However, there are few studies about the higher-level phylogeny of Tenebrionoidea based on mitogenomes. *Timmermans et al. (2010*, *2016)* used mitogenomes to explore the high-level phylogeny of Coleoptera, and their results suggested that Tenebrionoidea was monophyletic. *Zhang et al. (2016)* used ten mitogenomes of six subfamilies in Tenebrionidae to analyze the phylogenetic relationship of its subfamilies, and their results suggested that Lagriinae and Pimeliinae were sister groups, but that the relationships of Diaperinae and Tenebrioninae were inconclusive. Subsequently, *Song et al. (2019)* described the characteristics of the *Gonocephalum outreyi* mitogenome and reconstructed the higher-level relationship of Tenebrionoidea based on 26 tenebrionoid mitogenomes, showing that Tenebrionoidea is monophyletic, Tenebrionidae and Ciidae are sister groups, and Diaperinae and Tenebrioninae were non-monophyletic. Finally, *Wu et al. (2022)* reported on the characteristics of Alleculinae mitogenomes and reconstructed the high-level relationship of Tenebrionidae based on 36 mitogenomes, suggesting that Lagriinae, Pimeliinae, Stenochiinae, and Alleculinae were monophyletic, but that Diaperinae and Tenebrioninae were polyphyletic. Recently, genome data were also used to explore the phylogeny of beetles (*McKenna et al., 2019*; *Cai et al., 2022*).

In the present study, we sequenced and annotated the entire mitogenome sequences of four Lagriinae species, namely, *Cerogira janthinipennis* (Fairmaire, 1886), *Luprops yunnanus* (Fairmaire, 1887), *Anaedus unidentasus* Wang & Ren, 2007, and *Spinolyprops cribricollis* Schawaller, 2012. Of these, two tribes (Goniaderini and Lupropini) are the first to have their complete mitogenomes sequenced. We also conducted a preliminary analysis of genetic compositions and structural alterations to gain a better understanding of the phylogenetic relationships of Tenebrionoidea. Three newly-sequenced mitogenomes were provided (*Crypsis chinensis* Kaszab, 1946, *Gonocephalum kochi* Kaszab, 1952 and *Morphostenophanes yunnanus* Zhou, 2020) and data were obtained from the NCBI database to examine the phylogenetic relationship among species in the family Tenebrionidae. Mitogenomic data from these four lagriine species, as well as the evolutionary relationships within the subfamily Lagriinae, were reported in this study. The results of this work will provide a valuable basis for further evolutionary studies on beetles of Tenebrionoidea.

MATERIALS AND METHODS

Sampling and DNA extraction

Cerogira janthinipennis and *Crypsis chinensis* specimens were collected in the Dayaoshan Mountains of the Guangxi Zhuang Autonomous Region, China. *Luprops yunnanus* and *Spinolyprops cribricollis* were collected using the sieve soil method in Jinghong City, Yunnan Province, China. A specimen of *A. unidentasus* was collected using the same method in Shiqian County, Guizhou Province, China. *Gonocephalum kochi* specimens were collected in Zunyi City, Guizhou Province, China, and *Morphostenophanes yunnanus* were collected in Yunlong County, Yunnan Province, China. The leg and thorax tissues were immediately preserved in 95% ethanol in the field and then were stored at −24 °C. The specimens were identified based on morphological characteristics. Total DNA was extracted from the muscle tissues using the Ezup Column Animal Genomic DNA Purification Kit (Shanghai, China) in accordance with the manufacturer's instructions. This study was conducted under the approved guidelines of Animal Care and Use Committee of China West Normal University (approval number CWNU2022D021).

Sequencing, sequence assembly, annotation, and analysis

All of the mitogenomes in this study were sequenced with a whole genome shotgun strategy and Illumina sequencing technology using 150 bp paired-end reads. The adapter sequence and low-quality bases were deleted using Trimmomatic v. 0.36 (*Bolger, Logse & Usadel, 2014*). The target reads were assembled using IDBA UD v. 1.1.1 and Celera Assembler v. 8.3 (*Crampton-Platt et al., 2015*). The *de novo* assembly of the newly-generated sequences was conducted using Geneious 11.0.2 (*Kearse et al., 2012*). Protein-coding genes (PCGs) were identified as open reading frames. A total of 22 transfer RNA genes (tRNAs) were identified with the use of the MITOS Webserver, setting the default parameters with the Invertebrate Mito genetic code (*Bernt et al., 2013*). Their secondary structures were plotted manually from the MITOS predictions using Adobe Illustrator. Every sequence of tRNA genes was manually checked. The ribosomal RNA genes (rRNAs) and control region were identified by the boundaries of the tRNA genes. Mitogenome maps were produced using Organellar Genome DRAW COGDRAW (*Greiner, Lehwark & Bock, 2019*). The base composition and relative synonymous codon usage values of four Lagriinae mitogenomes were calculated using MEGA v 6.0 (*Kumar, Stecher & Tamura, 2016*). Strand asymmetry was calculated through the formula provided by *Perna & Kocher (1995)*: AT-skew = (A − T)/(A + T), GC-skew = (G − C)/(G + C). The non-synonymous substitutions (Ka), synonymous substitutions (Ks), and Ka/Ks of PCG genes were calculated using DnaSP v. 5 (*Rozas et al., 2003*). Tandem repeat units in the control region of four Lagriinae species were identified using the Tandem Repeats Finder online tool (*Benson, 1999*).

Phylogenetic analyses

Phylogenetic trees for *C. janthinipennis*, *L. yunnanus*, *A. unidentasus*, *S. cribricollis*, and other tenebrionid beetles were reconstructed by aligning sequences of mitogenomes with those of 39 tenebrionid species (Table S1). *Lymexylon navale* and *Hyloecetus dermestoides*,

belonging to Lymexyloidea, were chosen as outgroups, as they are phylogenetically closed to Tenebrionoidea in the Coleoptera (*Timmermans et al., 2016*; *Cai et al., 2022*). Phylogenetic trees were constructed using PhyloSuite v. 1.2.2 (*Zhang et al., 2020*) based on the maximum likelihood (ML) and Bayesian inference (BI) methods, respectively. The default parameters (ML: bootstrap: ultrafast; number of bootstrap: 5,000; maximum iterations: 1,000; minimum correlation coefficient: 0.90; replicates: 1,000; BI: generations: 2,000,000; sampling frequency: 100; number of runs: two; number of chains: four; burn-in fraction: 0.25) were used to construct phylogenetic trees. The default parameters (ML: bootstrap: ultrafast; number of bootstrap: 5,000; maximum iterations: 1,000; minimum correlation coefficient: 0.90; replicates: 1,000; BI: generations: 2,000,000; sampling frequency: 100; number of runs: two; number of chains: four; burn in fraction: 0.25) were used to construct phylogenetic trees. The resulting phylogenetic trees were edited and visualized using FigTree v. 1.43 (*Horton et al., 2015*).

RESULTS

Genome organization and base composition

We sequenced and annotated the complete mitogenomes of four lagriine species *Cerogira janthinipennis* (ON303727, length: 15,328 bp), *Luprops yunnanus* (ON303728, length: 16,437 bp), *Anaedus unidentasus* (ON303730, length: 15,387 bp), and *Spinolyprops cribricollis* (ON303729, length: 15,454 bp), as well as three other tenebrionid species: *Gonocephalum kochi* (ON303729, length: 15,825 bp), *Crypsis chinensis* (ON303729, length: 16,724 bp), and *Morphostenophanes yunnanus* (MW822745, length: 15,616 bp). The complete mitogenomes of these seven species contained 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and a control region (CR). Four of the 13 PCGs (*nad1*, *nad4L*, *nad4*, *nad5*), two rRNAs, and eight of the 22 tRNAs (*trnY*, *trnC*, *trnQ*, trn*V*, *trnL1*, *trnP*, *trnH*, *trnF*) are encoded on the N-strand, while the other 23 genes (nine PCGs and 14 tRNAs) are encoded on the J-strand (Table S1; Figs. S1, S2). The mitogenome sequences of these four lagriine species were determined to be medium- to maximum-sized compared to those of the Tenebrionidae species as a whole, which ranged from 15,328 bp (*C. janthinipennis*) to 16,437 bp (*L. yunnanus*).

The AT nucleotide contents of the four lagriine mitogenomes were similar (79.81% in *C. janthinipennis*, 72.12% in *L. yunnanus*, 74.90% in *A. unidentasus*, and 75.43% in *S. cribricollis*). The entire mitogenome had high A + T contents (ranging from 72.12–79.81%; 69.86–78.54% for PCGs, 77.34–81.16% for tRNAs, 76.79–82.56% for rRNAs, and 77.62–87.33% for the CR) (Table 1). The AT-skews in the four mitogenome sequences were −0.048, 0.127, 0.091, and 0.044, respectively, of which the *C. janthinipennis* had a negative value. All of the GC skews were negative (Table 1), which indicated that the content of G is lower than C.

Protein-coding regions and codon usage

In these four lagriine complete mitogenomes, the lengths of PCGs ranged from 11,030 to 11,087 bp, accounting for 67.36–72.22% of the full mitogenomes. In 13 PCGs, the *nad5*

Table 1 Nucleotide composition of four newly determined Lagriinae mitogenomes. The order of the values of the four species in the table are separated by/as follows: *Cerogira janthinipennis, Luprops yunnanus, Anaedus unidentasus, Spinolyprops cribricollis*. - not determined.

Gene	Strand	Position from	To	Start codons	Stop condons	Intergenic nucleotides
trnI	J	1/1/1/1	62/63/66/63			0/0/0/0
trnQ	N	60/61/68/64	128/129/136/132			-3/-3/1/0
trnM	J	129/130/136/132	191/198/199/194			0/0/-1/-1
nd2	J	192/199/200/195	1,196/1,203/1,195/1,187	ATT/ATA/ATT/ ATT	TAA/TAA/TAG/ TAA	0/0/0/0
trnW	J	1,195/1,202/1,228/1,191	1,257/1,267/1,294/1,254			-2/-2/32/3
trnC	N	1,258/1,267/1,300/1,254	1,318/1,327/1,360/1,313			0/-1/5/-1
trnY	N	1,319/1,328/1,365/1,315	1,381/1,391/1,430/1,378			0/0/4/1
cox1	J	1,383/1,393/1,432/1,380	2,916/2,923/2,962/2,910	-/-/-/-	TAA/TAA/TAA/ TAA	1/1/1/1
trnL2	J	2,917/2,924/2,963/2,911	2,979/2,986/3,025/2,973			0/0/0/0
cox2	J	2,980/2,987/3,026/2,974	3,660/3,665/3,704/3,652	ATT/ATA/ATA/ ATA	TAG/TAA/TAA/ TAA	0/0/0/0
trnK	J	3,662/3,666/3,705/3,653	3,731/3,735/3,774/3,722			1/0/0/0
trnD	J	3,731/3,736/3,777/3,727	3,794/3,799/3,839/3,789			-1/0/2/4
atp8	J	3,795/4,326/3,842/3,847	3,953/4,481/4,033/3,993	ATT/ATT/ATA/ ATA	TAA/TAG/TAA/ TAA	0/526/2/55
atp6	J	3,947/4,475/4,027/3,987	4,616/5,146/4,695/4,655	ATG/ATG/ATG/ ATG	TAA/TAA/TAA/ TAA	-7/-7/-7/-7
cox3	J	4,617/5,146/4,695/4,655	5,399/5,929/5,480/5,438	ATG/ATG/ATG/ ATG	TAA/TAA/TAA/ TAA	0/-1/-1/-1
trnG	J	5,401/5,930/5,480/5,439	5,462/5,992/5,541/5,500			1/0/-1/0
nad3	J	5,463/5,993/5,542/5,501	5,816/6,346/5,895/5,854	ATA/TTG/ATT/ ATA	TAG/TAG/TAG/ TAG	0/0/0/0
trnA	J	5,815/6,345/5,894/5,853	5,866/6,409/5,956/5,915			-2/-2/-2/-2
trnR	J	5,877/6,409/5,956/5,915	5,939/6,472/6,019/5,977			10/-1/-1/-1
trnN	J	5,939/6,472/6,019/5,977	6,004/6,537/6,081/6,040			-1/-1/-1/-1
trnS1	J	6,005/6,538/6,082/6,041	6,063/6,595/6,138/6,099			-1/-1/-1/-1
trnE	J	6,064/6,596/6,139/6,100	6,125/6,657/6,201/6,161			0/0/0/0
trnF	N	6,124/6,656/6,200/6,160	6,186/6,717/6,262/6,221			0/0/0/0
nd5	N	6,187/6,718/6,262/6,222	7,891/8,416/7,968/7,926	ATA/ATT/ATT/ ATT	TAA/TAA/TAA/ TAA	0/0/-1/0
trnH	N	7,892/8,417/7,972/7,927	7,957/8,484/8,033/7,988			0/0/3/0
nad4	N	7,958/8,485/8,033/7,989	9,284/9,811/9,358/9,312	ATG/ATG/ATG/ ATG	TAA/TAA/TAA/ TAA	0/0/-1/0
nad4L	N	9,278/9,805/9,352/9,306	9,565/1,0092/9,627/9,584	ATG/ATG/ATT/ ATA	TAA/TAA/TAA/ TAA	-7/-7/-7/-7
trnT	J	9,568/10,098/9,630/9,587	9,631/10,160/9,692/9,649			2/5/2/2
trnP	N	9,632/10,161/9,693/9,650	9,693/10,223/9,756/9,711			0/0/0/0

(Continued)

Table 1 (continued)

Gene	Strand	Position from	To	Start codons	Stop condons	Intergenic nucleotides
nad6	J	9,697/10,226/9,759/9,714	10,179/10,717/10,247/10,202	ATG/ATC/ATC/ATA	TAA/TAA/TAA/TAA	3/2/2/2
cytb	J	10,179/10,717/10,247/10,202	11,315/11,850/11,377/11,332	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	-1/-1/-1/-1
trnS2	J	11,314/11,849/11,377/11,331	11,380/11,915/11,440/11,394			-2/-2/-1/-2
nad1	N	11,398/12,105/11,469/11,415	12,348/13,055/12,419/12,359	TTG/TTG/TTG/TTG	TAG/TAG/TAG/TAA	17/89/28//10
trnL1	N	12,349/13,056/12,420/12,360	12,410/13,117/12,482/12,422			0/0/0/0
rrnL	N	12,411/13,118/12,483/12,423	13,675/14,395/13,750/13,685			0/0/0/0
trnV	N	13,676/14,396/13,751/13,686	13,740/14,464/13,813/13,752			0/0/0/0
rrnS	N	13,741/14,465/13,814/13,753	14,436/15,168/14,556/14,486			0/0/0/0
A+T rich region		14,437/15,169/14,557/14,487	15,328/16,437/15,387/15,454			0/0/0/0

(1,699–1,707 bp) and *atp8* (147–192 bp) were the largest and smallest genes, respectively. All the PCGs had a typical ATN start codon, except *nad1*, which began with TTG. For stop codons, most PCGs terminated with TAR (TAA/TAG) and few had an incomplete stop codon T- (the TAA stop codon is completed by the addition of 3′ A residues to the mRNA) (Table S1).

In PCGs, the numbers of amino acids and relative synonymous codon usage (RSCU) were calculated. The 13 PCGs of *A. unidentasus*, *C. janthinipennis*, *L. yunnanus*, and *S. cribricollis* contained 3,684, 3,682, 3,681, and 3,667 codons, respectively, excluding stop codons. In these four species, the most frequently used codons were UUU (276, 379, 224, 280), UUA (299, 427, 274, 348), AUU (334, 401, 266, 350), and AUA (223, 243, 200, 231) (Fig. 1). Accordingly, F, L2, I, and N are the most frequently used amino acids in these four species.

The ratio of nonsynonymous/synonymous (Ka/Ks) of 13 PCGs was also calculated; the result is representative of mutations and the evolutionary rate (*Hurst, 2002*). The Ka/Ks of *nad1* (3.157) are distinctly higher than 1 (Fig. 2), indicating that it is under strong positive selection (*Mori & Matsunami, 2018*), whereas *cox1* (0.088), *cox2* (0.114), and *cytb* (0.129) had values lower than the other genes (Fig. 2). These ratios suggest that the *cox1*, *cox2*, and *cytb* genes can be used as barcodes for deducing the phylogenetic relationships of Tenebrionidae.

In PCGs, the nucleotide diversity (Pi) with different data ranged from 0.211 (*cox1*) to 0.978 (*atp8*) (Fig. 3). Among the 13 PCGs, the gene *atp8* (Pi = 0.978) is the most diverse

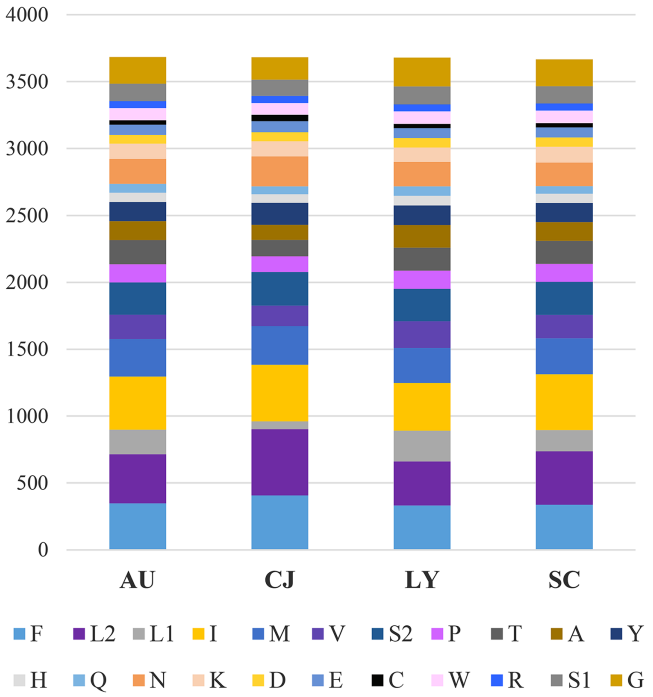

Figure 1 Numbers of different amino acids in the mitogenomes of four lagriine species; the stop codon is not included. Abbreviations: AU, *Anaedus unidentasus*; CJ, *Cerogira janthinipennis*; LY, *Luprops yunnanus*; SC, *Spinolyprops cribricollis*. Full-size DOI: 10.7717/peerj.15483/fig-1

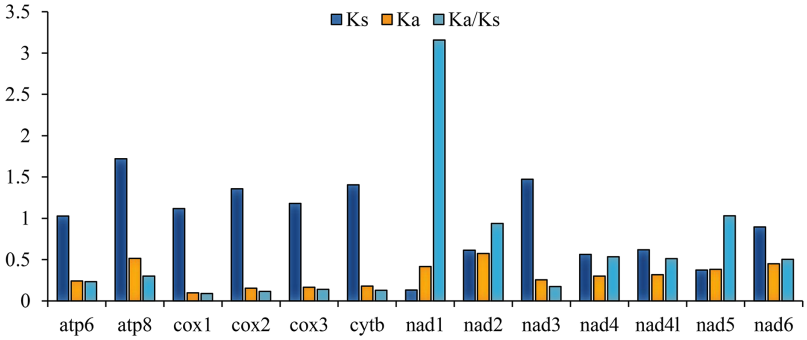

Figure 2 The ratio of Ka/Ks of 13 PCGs among the four lagriine mitogenomes. Full-size DOI: 10.7717/peerj.15483/fig-2

nucleotide, followed by *nad2* (Pi = 0.407) and *nad6* (Pi = 0.375), however, *cox1* is the most conserved gene with the lowest value (Pi = 0.211).

Transfer and ribosomal RNA genes

The total tRNAs lengths of *A. unidentasus*, *C. janthinipennis*, *L. yunnanus*, and *S. cribricollis* were 1,403, 1,391, 1,421, and 1,395 bp, respectively. Eight of the 22 tRNAs (*trnY*, *trnC*, *trnQ*, *trnV*, *trnL1*, *trnP*, *trnH*, *trnP*) were encoded on the N-strand, while the other 14 tRNAs were on the J-strand (Table 1). The tRNAs of four species were between 52 and 70 bp in length. The *trnK* is the longest of four species, while *trnS1*, *trnA*, *trnS1*, and *trnS1* are the shortest of *A. unidentasus*, *C. janthinipennis*, *L. yunnanus*, and *S. cribricollis*,

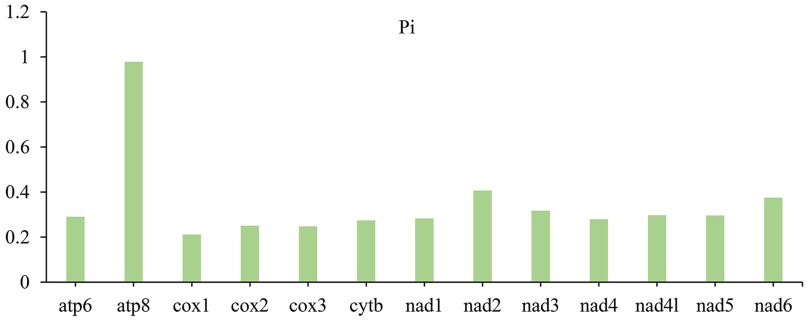

Figure 3 **Nucleotide diversity (Pi) of 13 PCGs among four lagriine mitogenomes.**
Full-size DOI: 10.7717/peerj.15483/fig-3

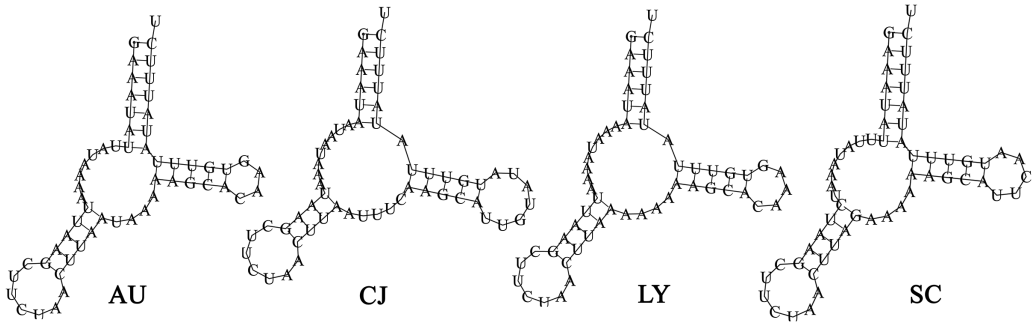

Figure 4 **The predicted secondary structures of the *trnS1* in the mitogenomes of the four lagriine species.** Abbreviations: AU, *Anaedus unidentasus*; CJ, *Cerogira janthinipennis*; LY, *Luprops yunnanus*; SC, *Spinolyprops cribricollis*.
Full-size DOI: 10.7717/peerj.15483/fig-4

respectively. Among the 22 tRNAs, 21 tRNAs showed the typical clover-leaf secondary structure (Figs. S2–S5), however, *trnS1* had a simple loop (Fig. 4). In *L. yunnanus*, a 526 bp insertion was present at *trnD* and *atp8* junction.

The total length of rRNAs ranged from 1,961 bp (*C. janthinipennis*) to 2,011 bp (*A. unidentasus*). The AT content of four species ranged from 76.79% (*L. yunnanus*) to 82.56% (*C. janthinipennis*) (Table 2). The two rRNAs (*rrnL* and *rrnS*) of four species are encoded on the N-strand. The rRNAs of four species were located between the control region and *trnL1*, and separated by *trnV*.

Control region

The control region (CR) was the longest non-coding region. It was located between *trnI* and *rrnS*. The length of CR ranged from 831 bp (*A. unidentasus*) to 1,269 bp (*L. yunnanus*). The AT content of the four species ranged from 77.62% (*L. yunnanus*) to 87.33% (*C. janthinipennis*), which is distinctly higher than that of the full mitogenome, PCGS, rRNAs, and tRNAs in the same species. These four sequences had a positive AT skew. The tandem repeat element numbers of *A. unidentasus*, *C. janthinipennis*, *L. yunnanus*, and *S. cribricollis* are 1, 5, 3, and 3, respectively.

Table 2 Nucleotide composition of four newly determined mitogenomes.

Species	Full mitogenome						PCGs	rRNAs	tRNAs	CR
	Length (bp)	A%	C%	A + T%	AT-skew	GC-skew	A + T%	A + T%	A + T%	A + T%
Cerogira janthinipennis	15,328	38.00	10.49	79.81	−0.048	−0.039	78.54	82.56	81.16	87.33
Luprops yunnanus	16,437	40.65	18.12	72.12	0.127	−0.300	69.86	76.79	77.34	77.62
Anaedus unidentasus	15,387	40.85	16.77	74.90	0.091	−0.336	73.33	79.41	77.62	80.99
Spinolyprops cribricollis	15,454	39.37	16.29	75.43	0.044	−0.326	73.75	78.97	78.42	82.33

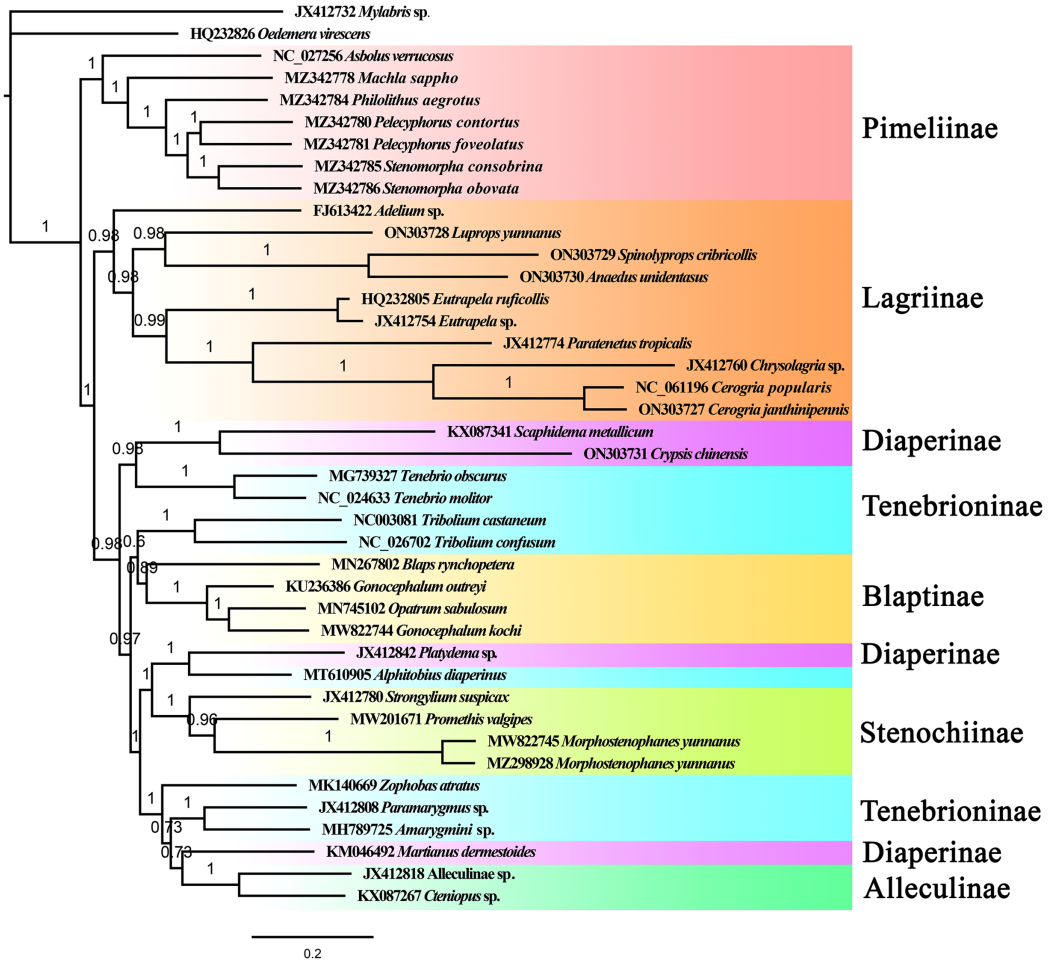

Figure 5 **The BI tree of Tenebrionidae inferred based on 13 PCGs.** The value on each branch shows its posterior probability. Full-size DOI: 10.7717/peerj.15483/fig-5

Phylogenetic analyses

The phylogenetic relationships among 39 Tenebrionidae species (Table S1) were reconstructed based on the nucleotide sequences of mitogenomes using the maximum likelihood and Bayesian inference methods. The outgroup species, *Mylabris* sp. (Meloidae) and *Oedemera virescens* (Oedemeridae), were the first to be separated from the tenebrionid clade. Phylogenetic trees based on the ML and BI methods showed approximately identical

topologies (Figs. 5, S10). All the Tenebrionidae species were clustered together, which suggested that Tenebrionidae is monophyletic. The target species (*A. unidentasus*, *C. janthinipennis*, *L. yunnanus*, and *S. cribricollis*) and other lagriine species were clustered into single branch with high support (Fig. 5, BI = 0.87) suggesting that Lagriinae is monophyletic. These results are consistent with previous studies (*Doyen & Tschinkel, 1982*; *Doyen, Matthews & Lawrence, 1989*; *Kergoat et al., 2014*; *Gunter et al., 2014*; *Timmermans et al., 2016*; *Aalbu, Kanda & Smith, 2017*; *Wu et al., 2022*). The three other target species, *Crypsis chinensis*, *Gonocephalum kochi*, and *Morphostenophanes yunnanus*, were clustered together with Diaperinae, Blaptinae, and Stenochiinae species, respectively. In this study, the monophyly of the subfamilies Pimelinae (BI = 1), Blaptinae (BI = 0.89), Stenochiinae (BI = 1), Alleculinae (BI = 1) was also supported (Fig. 5); the subfamily Diaperinae was paraphyletic, and Tenebrioninae appears polyphyletic. The subfamily Pimelinae was the sister group to the remaining subfamilies, which was consistent with the results of *Kergoat et al. (2014)* but inconsistent with the results of other studies (*Doyen & Tschinkel, 1982*; *Gunter et al., 2014*; *Timmermans et al., 2016*; *Wu et al., 2022*). The results also suggested that the classification of Tenebrioninae and Diaperinae needs to be further studied.

DISCUSSION

The genes composition and arrangement of four Lagriinae species were found to be the same as those in most other Tenebrionidae species (*Liu & Wang, 2014*; *Rider, 2016*; *Song et al., 2019*; *Yang et al., 2019*; *Hong et al., 2020*; *Bai et al., 2018*, *2019*, *2021*; *Smith et al., 2021*; *Liu et al., 2022*; *Wu et al., 2022*). In these four lagriine species, all complete mitogenomes had high A + T contents, which is consistent with the typical base bias of the Tenebrionidae mitogenomes. Compared to the subfamily Alleculinae (*Wu et al., 2022*), all PCGs of the subfamily Lagriinae had typical start codons and the ratio of Ka/Ks was lower than 1. In this study, the CR was located between *trnI* and *rrnS*, which is consistent with previous studies (*Liu & Wang, 2014*; *Rider, 2016*; *Song et al., 2019*; *Yang et al., 2019*; *Hong et al., 2020*; *Bai et al., 2021*; *Wu et al., 2022*).

In the subfamily Lagriinae, the genera *Spinolyprops* (Lupropini) and *Anaedus* (Goniaderini) formed a single clade; the results of *Aalbu, Kanda & Smith (2017)* suggested that the tribe Lupropini was paraphyletic, which was consistent with our findings. Based on the BI and ML tree topologies, the position of the genus *Spinolyprops* in the tribe Lupropini was not suitable. In terms of morphology, the genus *Spinolyprops* is more consistent with Goniaderini (*Matthews & Lawrence, 2019*). This is supported by the following characteristics: (1) concealed clypeolabral membrane; (2) pronotum with sides explanate; and (3) defensive glands absent (adult species from China without defensive glands). Based on mitogenome data and morphological characteristics, we recommend that the genus *Spinolyprops* should be transferred from Lupropini to Goniaderini. However, more samples are needed to confirm the present results. Compared to the tribes Lupropini, Goniaderini, and Lagriini, the tribe Adeliini is an original group.

In this study, the subfamily Tenebrioninae and Diaperinae were polyphyletic and paraphyletic, respectively. In the subfamily Tenebrioninae, the genus *Tenebrio* was closed to Diaperinae (*Scaphidema* + *Crypsis*). In the subfamily Diaperinae, the genus *Platydema*

and the genus *Alphitobius* (Tenebrioninae) were sister groups. These results were consistent with a previous study by *Wu et al. (2022)*. This phylogenetic question needs to be further addressed with more samples and more genes, as well as mitogenomic and nucleotide genes.

In the future, more mitogenome samples are needed to resolve the phylogeny of the Tenebrionidae to better understand the natural evolutionary processes.

CONCLUSION

In this study, the mitogenomes of the tribes Goniaderini and Lupropini were first reported and the compositional features were analyzed. The mitogenomes were 15,328–16,437 bp in length and encoded 37 typical mitochondrial genes. Comparisons of the four newly generated lagriine mitogenome to all available mitogenomes of Tenebrionidae revealed no significant differences among them in terms of the AT content of different genome regions, amino acid composition, and relative synonymous codon usage. In the PCGs, the *atp8* (Pi = 0.978) was the most diverse nucleotide, while *cox1* was the most conserved gene with the lowest value (Pi = 0.211). The phylogenetic results suggest that Pimelinae, Lagriinae, Blaptinae, Stenochiinae, and Alleculinae are monophyletic, Diaperinae is paraphyletic, and Tenebrioninae appears polyphyletic. In Lagriinae, the tribe Lupropini appears paraphyletic because *Spinolyprops* is clustered with *Anaedus* in Goniaderini. These mitogenome sequences provide valuable molecular data for the phylogenetic studies of Tenebrionidae in the future.

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The authors declare that they have no competing interests.

Author Contributions

- Zhonghua Wei conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Aimin Shi analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

This study was conducted 91 under the approved guidelines of Animal Care and Use Committee of China West Normal university (approval number CWNU2022D021).

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

Four new complete mitochondrial genomes are available at GenBank: ON303727, ON303728, ON303729, ON303730, and at SRA: PRJNA888200, PRJNA888252, PRJNA889942, PRJNA890044.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.15483#supplemental-information.

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