

The Complete Mitochondrial Genome of *Carabus davidis* (Coleoptera: Carabidae)

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ABSTRACT

Carabus davidis (Coleoptera: Carabidae) is a Category II protected species in China. In this study, we sequenced and annotated the complete mitochondrial genome of *C. davidis*, which is 16,624 bp in length and contains the typical set of 37 mitochondrial genes, including 13 protein-coding genes (PCGs), 22 transfer RNAs, two ribosomal RNAs, and a control region. The mitogenome shows a pronounced nucleotide compositional bias toward adenine and thymine, with an overall A+T content of 77.95%. Most PCGs had ATN start codons and TAN stop codons, except *nad5* (TTG start) and *cox2* (incomplete T-stop). All tRNAs had cloverleaf structures, except *trnSI*(AGN) (lacking a DHU arm), with 26 G-U mismatches. Phylogenetic and time-calibrated analyses indicate that *Carabus davidis* forms a well-supported, distinct lineage that diverged from its closest relatives during the early to middle Miocene. These results provide valuable genomic resources and evolutionary insights that contribute to the conservation and management of this protected species.

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Authors' Contribution

JF and XL conceived and designed the study. XL did methodology, formal analysis, original draft and visualization. SW carried out software and data curation. XL and XJ conducted investigation and material preparation. JF did resources, supervision, project administration, and funding acquisition. All authors contributed to review, editing, and approved the final manuscript.

Key words

Ground beetle, Mitogenome, Phylogenetic tree, Conservation

INTRODUCTION

Carabus davidis (Coleoptera: Carabidae), termed the “insect cheetah” for its agile movement, is listed as a National Second Class Protected Animal in China (Deyrolle and Fairmaire 1878). The taxonomy of genus *Carabus*, dating to the Linnaean era, has been repeatedly revised owing to its exceptional species richness, pronounced morphological disparity, and geographically structured subspecies (Ball, 1979), shifting from early morphology-based classifications (e.g., elytral venation, somatic architecture, integumentary pigmentation; Deuve, 2004) toward modern integrative frameworks that leverage molecular phylogenetics to resolve evolutionary relationships. Recognized as a highly specialized clade

within genus *Carabus*, *Carabus davidis* exhibits pronounced morphological and ecological divergence (Erwin, 1985). Its speciation history and current distribution reflect a strong phylogeographic signature shaped by major geological events, particularly Cenozoic orogenies and Pleistocene glacial–interglacial cycles, that have promoted allopatric fragmentation and lineage diversification across its range (Erwin, 1985).

Deep evolutionary relationships within the genus *Carabus* have long been controversial. Early morphological studies considered the subgenus *Apotomopterus* to represent the most primitive lineage (Breuning, 1932–1937), whereas subsequent classifications alternatively proposed *Spinulati* (Deuve, 1991, 1994) or *Digitulati* (Imura, 1996; Brezina, 1999) as the basal group. Based on the length and structural features of the short intergenic spacer between *ND5* and *tRNA^{Phe}*, Deuve (2004a) suggested *Arcifera* as sister to the remaining *Carabus*, a hypothesis later supported by molecular phylogenetic analyses showing a clade comprising *Arcifera* and *Spinulati* sister to all other congeners (Sota and Ishikawa, 2004). Nevertheless, conflicting signals and limited support at key nodes indicate that current morphological and molecular data remain insufficient to robustly resolve the deepest branching relationships within *Carabus*.

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In addition, *C. davidis* functions as an apex invertebrate predator within temperate forest ecosystems, thereby influencing detrital processing rates and soil ecosystem dynamics (Lövei and Sunderland, 1996; Su *et al.*, 1996). Owing to its limited dispersal capacity, narrow ecological niche breadth, and high sensitivity to habitat heterogeneity and environmental perturbation, *C. davidis* is widely regarded as a robust bioindicator taxon (Dufrêne *et al.*, 1990), and has been extensively employed in landscape-scale ecological studies to assess community-level responses of ground-dwelling carabid assemblages to anthropogenic drivers such as forest fragmentation, land-use intensification, and agricultural conversion (Eyre *et al.*, 1996; Negro *et al.*, 2007).

Due to its strict maternal inheritance, high substitution rate relative to nuclear DNA, and conserved gene order and structure, the mitochondrial genome has become a pivotal molecular marker for resolving phylogeographic patterns and phylogenetic relationships across both deep and shallow evolutionary timescales in insects (Wu *et al.*, 2014; Broughton *et al.*, 2001; Gong *et al.*, 2019). Based on a concatenated mitogenomic dataset, the monophyly of Carabidae and its sister-group relationship with Trachypachidae within Adephaga were strongly supported (Kieran, 2020). In contrast, a time-calibrated tribal phylogeny of Carabidae based on complete mitogenomes revealed that bursts of diversification coincided with Late Cretaceous–Paleogene orogeny and climate-driven habitat fragmentation vicariant drivers of allopatric speciation that shaped the current phylogeographic structure of carabid assemblages in temperate biomes (Lin *et al.*, 2024). Despite its ecological importance as a bioindicator and its relevance to conservation biogeography, *C. davidis* lacks a fully characterized mitochondrial genome, limiting high-resolution population genetic, phylogeographic, and comparative genomic analyses.

In this study, we sequenced, assembled, and annotated the complete mitochondrial genome of *C. davidis* from the Huangshan Scenic Area (Anhui Province, China), characterizing its genomic architecture, nucleotide composition, codon usage, and tRNA secondary structures. We further inferred its phylogenetic relationships within genus *Carabus* using the cytochrome b (*Cytb*) gene, providing foundational mitogenomic resources for future studies on molecular ecology, population genetics, and conservation management of this protected species.

MATERIALS AND METHODS

Sampling and DNA extraction

The *C. davidis* specimens used in this study were collected by the author from the Huangshan Scenic Area,

Anhui Province, China (30° 07' 15" N, 118° 10' 12" E). The specimens were preserved in absolute ethanol at −20 °C for subsequent molecular analysis. Genomic DNA was extracted from the ethanol-fixed tissues via the phenol/chloroform method. The resulting DNA was then quantified and qualified using 1% agarose gel electrophoresis (Guo *et al.*, 2021).

DNA sequencing and splicing

The genomic DNA library was prepared starting with 1 µg of DNA. The DNA was randomly sheared into fragments of 300–500 bp. Following fragmentation, end repair, A-tailing, and adapter ligation were performed using the Illumina TruSeq™ Nano DNA Sample Prep Kit (Illumina, San Diego, USA). The adapter-ligated DNA was then amplified with 8 cycles of PCR. The resulting PCR products were size-selected by 2% agarose gel electrophoresis, targeting fragments in the 300–500 bp range, and purified. Finally, paired-end sequencing (2 × 150 bp) was conducted on an Illumina NovaSeq 6000 platform at Beijing Qingke Biotechnology Co., Ltd.

Mitochondrial genome assembly and annotation

Raw reads were quality-controlled using Trimmomatic v0.39 (<http://www.usadellab.org/cms/index.php?page=trimmomatic>) (Bolger *et al.* 2014) under default parameters to remove adapter sequences, low-quality bases, and short fragments, yielding high-quality clean reads for downstream assembly. PacBio Sequel II sequencing data in BAM format were converted to FASTA format and subjected to stringent filtering to retain high-quality subreads longer than 200 bp, which provided long-range information for subsequent hybrid assembly.

A draft mitochondrial genome was first assembled from Illumina short reads using GetOrganelle (<https://github.com/Kinggerm/GetOrganelle>) (Jin *et al.* 2020). These draft sequences were aligned against PacBio (Rhoads and Au, 2015) long reads using BWA to extract mitochondrial-derived long fragments. A hybrid assembly was then performed with SPAdes (Prjibelski *et al.*, 2020), integrating accurate Illumina short reads and repetition-spanning PacBio long reads to generate longer contiguous sequences. The resulting assembly was compared against the NT (Bankevich *et al.*, 2012) database to confirm mitochondrial origin and polished with Pilon (Walker *et al.*, 2014) using high-quality clean reads to correct base-level errors, resulting in a complete and high-quality mitochondrial genome.

Analysis of mitochondrial genome characteristics

Gene annotation was performed using the MITOS web server (<http://mitos.bioinf.uni-leipzig.de/index.py>)

(Bernt *et al.*, 2013), identifying 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and non-coding control regions. Genome features were characterized by analyzing gene order, overlapping regions, and intergenic spacers. Nucleotide composition statistics including GC content, AT-skew, and GC-skew were calculated for the whole genome and specific functional regions. Additionally, start and stop codon usage in protein-coding genes was examined.

A standard annotation file in GenBank format was generated, and a circular genome map was visualized using CGView (http://stothard.afns.ualberta.ca/cgview_server/) (Grant and Stothard, 2008) to illustrate gene positions, transcription directions, and base composition characteristics.

Phylogenetic analyses

This study reconstructed the phylogenetic relationships within the genus *C.* based on the cytochrome b (*Cytb*) gene fragment of *Carabus davidis*, along with sequences from 13 closely related *Carabus* species retrieved from the GenBank database. *Calosoma sycophanta* was selected as the outgroup.

Phylogenetic trees were constructed using both the Neighbor-Joining (NJ) method in MEGA 11.0 (Tamura *et al.*, 2021) and bayesian inference (BI) in MrBayes v3.2 (Ronquist *et al.*, 2012) for the BI analysis, the Markov Chain Monte Carlo algorithm was run until convergence criteria were met, with the first 25% of samples discarded as burn-in. Branch support in the NJ analysis was assessed using 1,000 bootstrap replicates.

Divergence time estimation

Divergence time estimation for *Carabus* were conducted in BEAST 10.5.0 (Bouckaert *et al.*, 2019) using the mitochondrial cytochrome b gene as a phylogenetically informative molecular marker. An uncorrelated lognormal relaxed molecular clock model was implemented to accommodate lineage-specific rate heterogeneity across the phylogeny (Osozawa and Wakabayashi, 2020). A pure-birth process was specified as the tree prior to model speciation dynamics under a constant-rate diversification scenario. BI was performed via MCMC sampling over 20 million generations, with parameter states logged every 1,000 generations to ensure adequate effective sample sizes (ESS > 200) for all estimated parameters (Osozawa *et al.*, 2013). The initial 10% of samples were discarded as burn-in, and the post-burn-in posterior distribution of time-calibrated trees were summarized into a maximum clade credibility (MCC) tree using TreeAnnotator, with node heights represented by median posterior estimates and nodal support assessed via posterior probabilities.

Temporal calibration was implemented using two secondary calibration constraints derived from a previously published time-calibrated phylogeny of Carabinae (Andújar *et al.*, 2012). Specifically, (1) the divergence between *Carabus* (*Macrothorax*) *rugosus* and *C.* (*Macrothorax*) *morbillosus* was constrained with a lognormal prior centered on a mean age of 7.48 Ma and bounded by a 95% highest posterior density (HPD) interval of 6.05–9.14 Ma; and (2) the divergence between the subgenera *Eurycarabus* and *Nesaecarabus* was assigned a lognormal prior with a mean age of 9.51 Ma and a 95% HPD interval of 7.71–11.56 Ma.

RESULTS

Mitochondrial whole genome basic structure

The mitochondrial genome of *C. davidis* measured 16,624 bp (GenBank: PV288324) and represented a typical circular double-stranded DNA molecule. It comprised 37 genes, including 13 protein-coding genes (PCGs), 22 tRNA genes, 2 rRNA genes, and a control region (Fig. 1). Four PCGs (*nad1*, *nad4*, *nad4L*, *nad5*), eight tRNA genes (*tRNA^{Gln}*, *tRNA^{Cys}*, *tRNA^{Tyr}*, *tRNA^{Phe}*, *tRNA^{His}*, *tRNA^{Pro}*, *tRNA^{Leu(UAG)}*, *tRNA^{Val}*), and two rRNA genes (*rrnL*, *rrnS*) are encoded on the minority strand (N-strand), whereas the remaining nine PCGs (*nad2*, *nad3*, *nad6*, *cox1*, *cox2*, *cox3*, *cob*, *atp6*, *atp8*) and 14 tRNA genes (*tRNA^{Ile}*, *tRNA^{Met}*, *tRNA^{Trp}*, *tRNA^{Leu(UAA)}*, *tRNA^{Lys}*, *tRNA^{Asp}*, *tRNA^{Gly}*, *tRNA^{Ala}*, *tRNA^{Arg}*, *tRNA^{Asn}*, *tRNA^{Ser(CUU)}*, *tRNA^{Glu}*, *tRNA^{Thr}*, *tRNA^{Ser(UGA)}*) are encoded on the majority strand (J-strand). The genome exhibited eight overlapping regions (1–40 bp) and 23 intergenic spacers (1–214 bp) (Table I). The 1,488 bp control region was located between *rrnS* and *tRNA^{Ile}*. The overall mitochondrial genome structure of *C. davidis* aligns with that of existing Carabidae species (Kieran, 2020; Lin *et al.*, 2024).

Nucleotide composition and deviation analysis

The mitogenome of *C. davidis* has a nucleotide composition of 40.98% A, 36.98% T, 13.25% C, and 8.79% G, with a pronounced AT bias (A + T = 77.95%, G + C = 22.05%), which is consistent with that of other arthropods (Liu *et al.*, 2023; Wang *et al.*, 2023). The A + T content of transfer RNA (tRNA) genes is 77.49%, and that of ribosomal RNA (rRNA) genes is 80.83% (Table II). The control region is particularly rich in A + T, accounting for 85.41%. Among protein-coding genes (PCGs), the A + T content varies from 67.57% in *cox1* to 86.67% in *atp8*. The whole nucleotide chain was subjected to skew statistics (AT-skew = 0.051, GC-skew = -0.202). Except for most PCGs such as *nad1* (AT-skew = -0.269), *nad4* (AT-skew = -0.229), and *cox1* (AT-skew = -0.103) which

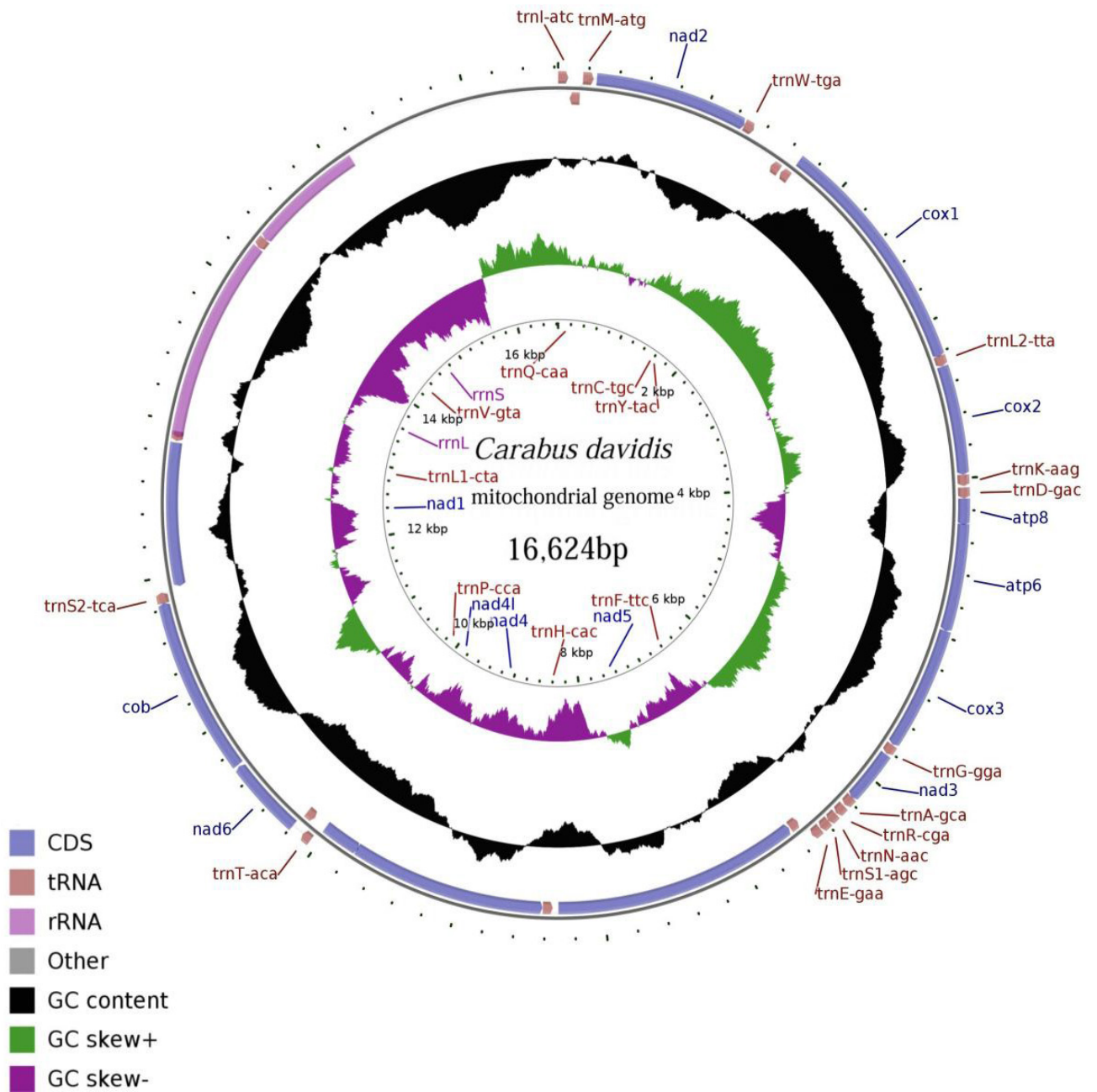


Fig. 1. Mitochondrial genome structure of *Carabus davidis*. The genes whose names are marked outside the circle are located on the H strand, while the rest of the genes are positioned on the L strand.

were negative, the AT-skew of tRNAs (AT-skew = 0.003) and rRNAs (AT-skew = -0.059) was close to zero or only slightly negative.

Notably, except for the tRNAs and the Control region genes exhibited negative AT skew values (T content > A

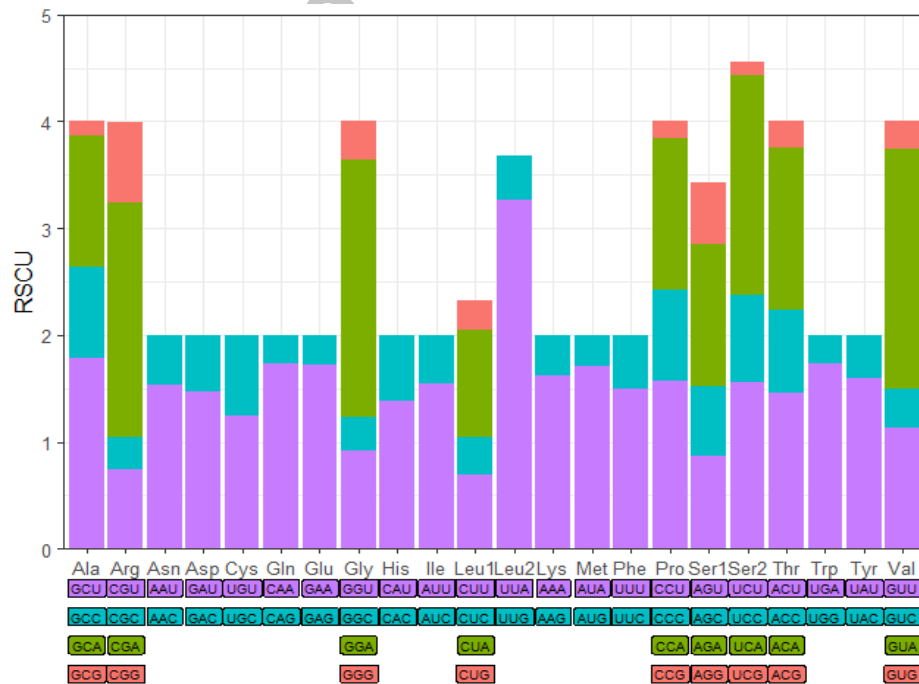
content). Except for *nad1* (GC-skew = 0.297), *nad4* (GC-skew = 0.280), and rRNAs (GC-skew = 0.345) which were positive, the GC-skew of the remaining majority of PCGs (such as *atp8*, *nad6*) and the whole genome was negative.

Table I. Organization of the mitochondrial genome of *Carabus davidis*.

Gene name	Coding strand	Start position	End position	Intergenic nucleotides	Overlapping nucleotide	Size (bp)	Start codon	Stop codon
<i>tRNA^{Ile}</i>	H	1	66	-	-	66	-	-
<i>tRNA^{Gln}</i>	L	79	147	12	-	69	-	-
<i>tRNA^{Met}</i>	H	164	232	16	-	69	-	-
<i>nad2</i>	H	254	1261	21	-	1008	ATT	TAA
<i>tRNA^{Trp}</i>	H	1263	1330	1	-	68	-	-
<i>tRNA^{Cys}</i>	L	1545	1609	214	-	65	-	-
<i>tRNA^{Tyr}</i>	L	1626	1691	16	-	66	-	-
<i>cox1</i>	H	1684	3228	-	8	1545	ATT	TAA
<i>tRNA^{Leu(UAA)}</i>	H	3224	3290	-	5	67	-	-
<i>cox2</i>	H	3292	3979	1	-	688	ATG	T
<i>tRNA^{Lys}</i>	H	3980	4050	-	-	71	-	-
<i>tRNA^{Asp}</i>	H	4060	4127	9	-	68	-	-
<i>atp8</i>	H	4128	4292	-	-	165	ATT	TAA
<i>atp6</i>	H	4286	4963	-	7	678	ATG	TAA
<i>cox3</i>	H	4963	5751	-	1	789	ATG	TAA
<i>tRNA^{Gly}</i>	H	5755	5820	3	-	66	-	-
<i>nad3</i>	H	5827	6174	6	-	348	ATT	TAG
<i>tRNA^{Ala}</i>	H	6173	6239	-	2	67	-	-
<i>tRNA^{Arg}</i>	H	6249	6315	9	-	67	-	-
<i>tRNA^{Asn}</i>	H	6321	6385	5	-	65	-	-
<i>tRNA^{Ser(CUU)}</i>	H	6386	6452	-	-	67	-	-
<i>tRNA^{Glu}</i>	H	6458	6524	5	-	67	-	-
<i>tRNA^{Phe}</i>	L	6545	6611	20	-	67	-	-
<i>nad5</i>	L	6619	8310	7	-	1692	TTG	TAA
<i>tRNA^{His}</i>	L	8350	8417	39	-	68	-	-
<i>nad4</i>	L	8420	9760	2	-	1341	ATG	TAA
<i>nad4l</i>	L	9754	10017	-	7	264	ATT	TAA
<i>tRNA^{Thr}</i>	H	10050	10113	32	-	64	-	-
<i>tRNA^{Pro}</i>	L	10114	10178	-	-	65	-	-
<i>nad6</i>	H	10196	10705	17	-	510	ATT	TAA
<i>cob</i>	H	10717	11841	11	-	1125	ATA	TAG
<i>tRNA^{Ser(UGA)}</i>	H	11840	11907	-	2	68	-	-
<i>nad1</i>	L	11924	12865	16	-	942	ATA	TAG
<i>tRNA^{Leu(UAG)}</i>	L	12876	12939	10	-	64	-	-
<i>rrnL</i>	L	12900	14271	-	40	1372	-	-
<i>tRNA^{Val}</i>	L	14274	14344	2	-	71	-	-
<i>rrnS</i>	L	14345	15137	-	-	793	-	-

Table II. Nucleotide composition of mitochondrial genome of *Carabus davidis*.

Regions	T (%)	C (%)	A (%)	G (%)	A+T (%)	AT skew	GC skew
Complete sequence	36.98	13.25	40.98	8.79	77.95	0.051314945	-0.202267574
tRNAs	38.64	9.69	38.85	12.81	77.49	0.002710027	0.138605064
rRNAs	42.77	6.28	38.06	12.89	80.83	-0.058270444	0.344809598
Control region	41.32	8.88	43.46	5.61	85.41	0.025055614	-0.224126114
atp6	41.45	15.34	34.07	9.14	75.52	-0.097722458	-0.253267974
atp8	43.64	10.9	43.03	2.42	86.67	-0.007038191	-0.63615904
cob	39.02	15.73	33.78	11.47	72.8	-0.071978022	-0.156617647
cox1	37.28	16.76	30.29	15.66	67.57	-0.103448276	-0.033919211
cox2	37.5	14.53	36.48	11.48	73.98	-0.01378751	-0.117217525
cox3	38.91	15.21	37.57	13.31	71.48	-0.018746503	-0.066619916
nad1	48.51	8.28	27.92	15.29	76.43	-0.269396834	0.297411964
nad2	42.56	11.51	38	7.94	80.56	-0.056603774	-0.183641975
nad3	43.1	12.07	35.06	9.77	78.16	-0.102865916	-0.105311355
nad4	48.25	7.31	30.28	14.17	78.52	-0.228858889	0.280228758
nad4l	56.06	6.06	27.27	10.61	83.33	-0.34549382	0.272945411
nad5	48.11	7.45	31.03	13.42	79.14	-0.215820066	0.286193672
nad6	44.51	10.2	39.8	5.49	84.31	-0.055865259	-0.300191205
rrnL	43.22	6.34	37.9	12.54	81.12	-0.065581854	0.328389831
rrnS	42	6.18	38.34	13.49	80.33	-0.045562057	0.371631927
First position	42.64	7.41	40.34	9.6	82.98	-0.027717522	0.128747795
Second position	40.83	13.3	29.82	16.03	70.65	-0.155838641	0.093047035
Third position	46.45	14.6	28.04	10.9	74.49	-0.247147268	-0.145098039

**Fig. 2. Relative synonymous codon usage (RSCU) of the mitogenomes of *Carabus davidis*.**

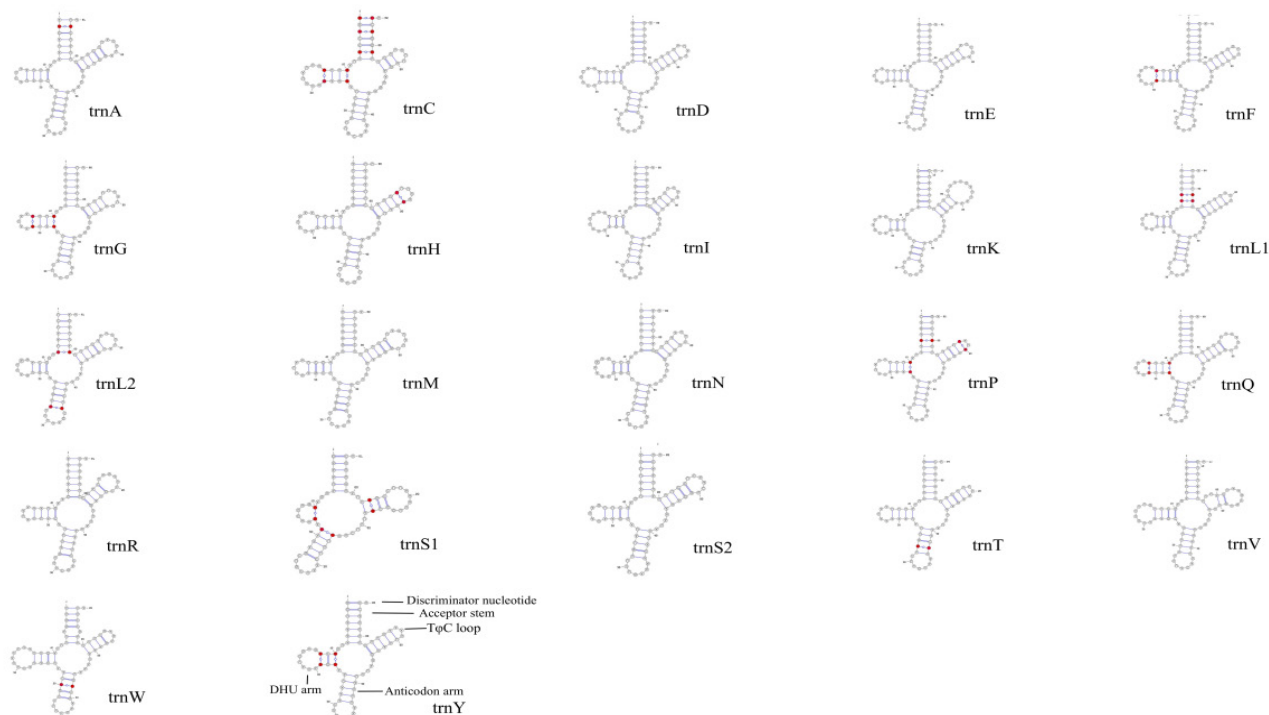


Fig. 3. The secondary structure predictions of tRNA genes of *Carabus davidis*. The standard base pairing bonds of A and T, and C and G were represented by "=", and the pairing bonds between G and U were indicated by "-".

PCGs and codon usage

Among the 13 protein-coding genes (PCGs), nine (*nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad6*, and *cob*) are located on the heavy strand (H-strand), while four (*nad5*, *nad4*, *nad4l*, and *nad1*) are encoded on the light strand (L-strand). All PCGs initiate with ATN start codons, with the majority using ATT (*nad2*, *atp8*, *nad3*, *nad4l*), ATG (*cox2*, *atp6*, *cox3*, *nad4*), or ATA (*cob*, *nad1*). The only exception is *nad5*, which starts with TTG.

However, this pattern differs from that of *Carabus smaragdinus monilifer* (Oh *et al.*, 2020), in which *nad1* initiates with TTG, and from *Carabus lafossei*, where all PCGs use ATN as the start codon (Liu *et al.*, 2018).

Analysis of synonymous codon usage revealed a strong preference for UUA (Leu), which showed the highest relative synonymous codon usage (RSCU= 3.27). Other frequently used codons with high RSCU values such as AUU (Ile, RSCU= 1.55), UUU (Phe, RSCU= 1.50), and AUA (Met, RSCU= 1.71) are composed exclusively of A and U, reflecting a pronounced AT bias. Notably, all codons with RSCU values above 2.0, including UUA (Leu, 3.27), GUA (Val, 2.24), GGA (Gly, 2.41), and CGA (Arg, 2.19), consistently contain A at the third codon position, further supporting a pattern of AT-biased codon selection. In the resulting protein sequences, leucine (Leu) is the

most abundant amino acid, followed by isoleucine(Ile), phenylalanine (Phe), and methionine (Met), consistent with the high frequency of their corresponding codons.

tRNA and rRNA

This mitochondrial genome contains a total of 22 tRNA genes, spanning a combined length of 1475 bp. Among these, eight are located on the light strand (L/minor strand) and fourteen on the heavy strand (H/major strand). The longest tRNAs, *trnK* and *trnV*, each consist of 71 bp, while the shortest including *trnN*, *trnT*, and *trnL1* range from 64 to 65 bp. The overall A+T content of the tRNAs is 77.49%, with a near-neutral AT skew (0.003) and a positive GC skew (0.139). With the exception of *trnS1*, which lacks a DHU arm, all tRNA genes display the typical cloverleaf secondary structure.

The genome also encodes two ribosomal RNA genes: *rrnL* (large subunit, 1,372 bp) and *rrnS* (small subunit, 793 bp). Both genes exhibit high A+T content (81.12% and 80.33%, respectively) with negative AT-skew values, and notably positive GC-skew values (0.328 and 0.372, respectively). This pronounced A+T bias and positive GC-skew are consistent with the overall nucleotide composition pattern observed across the mitochondrial genome.

Control region, non-coding, and overlapping areas

The control region of the mitochondrial genome of *C. davidis* is 1488 base pairs in length, located between *rrnS* and *trnI*. The contents of A, T, C and G bases are 43.78%, 41.63%, 8.94% and 5.65% respectively, and the content of A + T bases is 85.41%. This shows a significant AT base preference (Tables I and II). The mitochondrial genome of *C. davidis* contains 28 non-coding regions (also known as intron spacer regions), with lengths ranging from 1 base pair to 215 bp, among which the longest one is 215 bp and is located between *trnW* and *trnC*. Additionally, 7 gene overlaps were observed, with lengths ranging from 1 bp to 39 bp, and the longest one (39 bp) occurs between *trnL1* and *rrnL* (Fig. 1, Table I).

Phylogenetic analysis

In the Cytb-based phylogeny encompassing 18 *Carabus* species, *Carabus mirabilissimus* and *C. lafossei*, *Chaetomelas piochardi* and *C. impressus*, and *C. saulcyi* and *C. hemprichi* each formed reciprocally monophyletic sister pairs with maximal Bayesian posterior probability (PP = 1.0), providing robust support for their respective sister-group relationships, a topology congruent with

Liu's earlier phylogenetic hypotheses (Liu *et al.*, 2018). However, the *C. davidis* occupied a distinct phylogenetic lineage, a pattern potentially reflecting its ecological divergence associated with mesic, humus-rich forest habitats.

Divergence-time estimation analysis

The time-calibrated phylogeny (Fig. 4) indicates that the major lineages of *Carabus* diversified primarily during the Miocene. The split between *Carabus* (*Macrothorax*) *rugosus* and *C. (Macrothorax) morbillosus* occurred at approximately 7.18 Ma, consistent with the applied secondary calibration. The divergence between the subgenera *Eurycarabus* and *Nesaeocarabus* was estimated to have occurred in the middle Miocene.

Within the focal clade, *C. davidis* (this study) formed a distinct lineage and diverged from its closest relatives at approximately 16.6 Ma, suggesting an early to middle Miocene origin. Subsequent diversification within this clade mainly took place during the late Miocene to Pliocene, with several species pairs showing relatively recent divergence events.

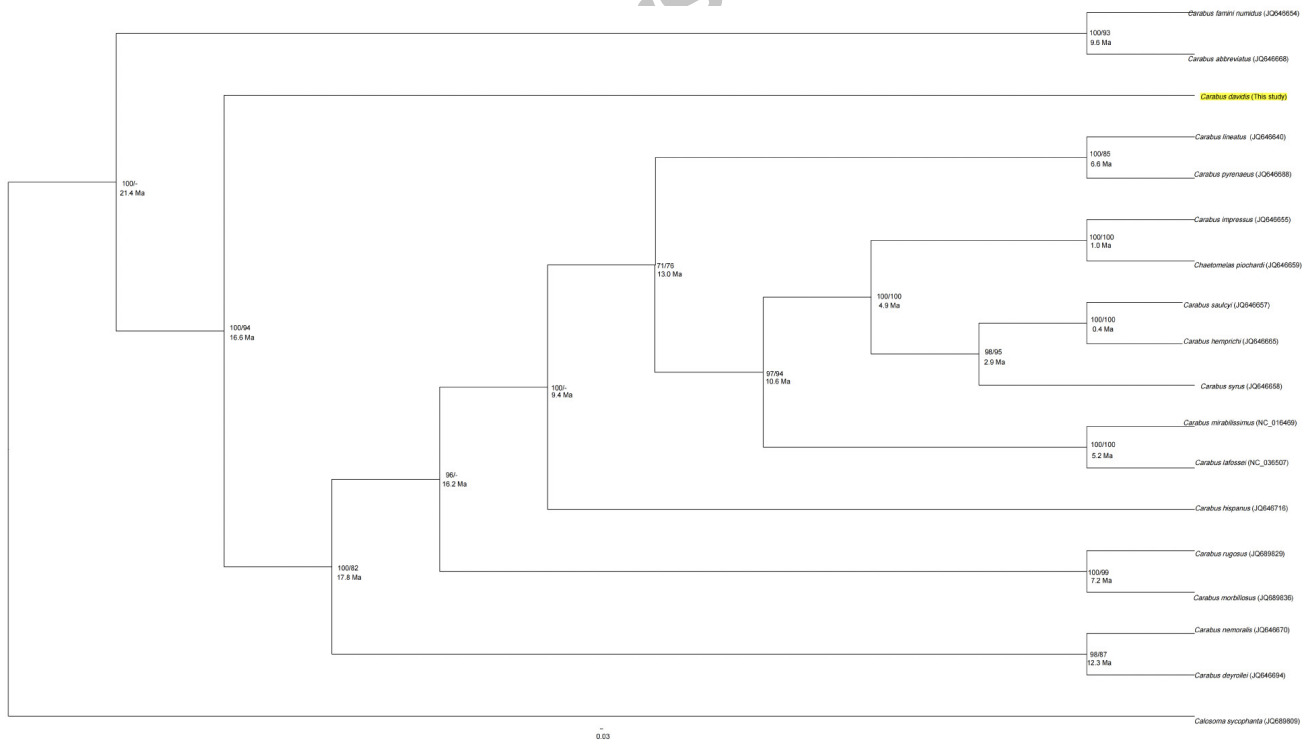


Fig. 4. Phylogenetic relationships of *Carabus* inferred from mitochondrial cytochrome b sequences. Bayesian posterior probabilities and Neighbor-Joining bootstrap percentages (BPP/NJB) are shown above the corresponding nodes, while estimated divergence times (Ma) are indicated below the nodes. *Carabus davidis* generated in this study is highlighted. *Calosoma sycophanta* was used as the outgroup.

DISCUSSION

This study presents the first complete mitochondrial genome of the protected species *C. davidis*. The mitochondrial genome is 16,624 bp in length, exhibits a typical circular structure, and its gene composition and sequence are consistent with most insects (Kwon *et al.*, 2023; Zhu *et al.*, 2025). A notable AT bias (77.95% A+T) was observed across the genome, particularly in the control region (85.41%), a pattern commonly reported in insect mitochondrial genomes (Crozier and Crozier, 1993). Most protein-coding genes use standard ATN initiation codons, with the notable exception of *nad5*, which starts with TTG a feature also present in related species such as *Carabus smaragdinus monilifer* (Oh *et al.*, 2020). Codon usage analysis further revealed a strong preference for A/T-ending codons, especially UUA (Leu), which had the highest RSCU value (3.27), reinforcing the overall nucleotide composition bias, a common characteristic of metazoans (Wang *et al.*, 2023; Liu *et al.*, 2023). Among the 22 tRNAs identified, all displayed the typical cloverleaf secondary structure except for *trnSI*(AGN), a similar situation occurs in other species of the Chlorogomphidae family. For example, *trnS1* of *Chlorogomphus papilio* also lacks a DHU arm (Jin *et al.*, 2025). Structural abnormalities in *trnS1*, like other mitochondrial genomic variations, arose early and have recurred frequently during metazoan evolution, including in insects (Crozier and Crozier, 1993), and these tRNAs with abnormalities are known to have less critical biological functions compared with normal tRNAs (Shi *et al.*, 2023; Hanada *et al.*, 2001). A total of 26 G–U mismatches were identified, which may be corrected by post-transcriptional modifications (Lavrov *et al.*, 2000; Negrisolo *et al.*, 2011).

Traditional classification systems for ground beetles are primarily based on external morphological characteristics, such as elytra carving, body proportions, and coloration. However, due to significant morphological convergence among ground beetle groups and continuous morphological variation among geographically isolated populations, this morphological-dependent classification method often introduces uncertainty when defining subgenus or species boundaries, especially in groups with highly specialized phylogenetic positions or ecosystems. This study, based on a phylogenetic tree reconstructed using complete mitochondrial genome structural features and *Cytb* gene sequences, provides molecular phylogenetic information independent of morphological evidence. The results show that *C. davidis* forms a stable and highly supported independent branch in the phylogenetic tree, clearly distinguishing it from its morphologically considered closely related groups. This molecular evidence suggests that *C. davidis* is not a “marginal member” as

inferred from some traditional classifications based on appearance similarities, but rather represents a lineage with clear evolutionary independence.

Divergence time estimation analysis indicates that genus *Carabus* originated approximately in the early to middle Miocene (16.7–25.1 Ma; Deuve *et al.*, 2012) with its main lineages diversifying rapidly around 10 Ma (6.6–14.8 Ma; Deuve *et al.*, 2012). During the 20–14 Ma, Eurasia was covered by extensive warm, humid forests dominated by thermophilous evergreen trees (Bruch *et al.*, 2007; Bruch and Zhilin, 2007), resembling modern subtropical to temperate forests in southeastern China (Jiménez-Moreno, 2006). These continuous forest landscapes probably enabled widespread dispersal and population expansion of forest-adapted *Carabus* lineages. Around 13 Ma, the Middle Miocene Climate Transition (MMCT; Escutia *et al.*, 2005) drove a biome shift in Eurasia from thermophilous evergreen forests to temperate deciduous woodlands (Agusti *et al.*, 2003; Eronen *et al.*, 2009), which expanding *Carabus* lineages appear to have tracked during their range expansion. Later in the Miocene, uplift of the Himalayas and Tibetan Plateau, together with regression of the Paratethys Sea, reconfigured atmospheric circulation and weakened East Asian monsoon (Zhisheng *et al.*, 2001). These geoclimatic changes triggered widespread aridification in Central Asia and promoted the expansion of steppe ecosystems (Quade *et al.*, 1989; Sun *et al.*, 1998; Hoorn *et al.*, 2000; Qiang *et al.*, 2001). The resulting habitat reorganization fragmented formerly continuous forests and isolated montane refugia, thereby restricting gene flow, promoting allopatric divergence, and shaping the present-day phylogeographic structure of *Carabus*. In summary, the assembly and annotation of the *C. davidis* mitogenome offer a valuable genomic resource for further studies on the molecular ecology, population genetics, and conservation strategies of this and related endangered insect species.

DECLARATIONS

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IRB approval

This study was approved by the Institutional Animal Care and Use Committee of Anhui University (Permit No.

IACUC(AHU)-2026-068).

Ethical statement

The collection and handling of *Carabus davidis* specimens were conducted following institutional guidelines, relevant national regulations, and the principles of the Convention on Biological Diversity. All efforts were made to minimize unnecessary harm and disturbance to the specimens during collection and preservation.

Data availability

The data presented in this study are available in NCBI GenBank (Accession number: PV288324).

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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