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The Complete Mitochondrial Genome of *Agrilus zanthoxylumi* Li, 1989 (Buprestidae: Agrilinae): Genome Characterization and Phylogenetic Implications

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ABSTRACT

Few mitogenomes sequences are available for *Agrilus* species in the family Buprestidae. To explore the mitochondrial genome features and their phylogenetic relationships, the complete mitogenome of the trunk borer jewel beetle, *Agrilus zanthoxylumi*, Li, 1989 (花椒窄吉丁) was sequenced and annotated. The complete 16,320 bp genome encodes 37 mitochondrial genes, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosomal RNA genes, and a 1526-bp-long AT-rich region. Most of the PCGs, except ND1 which uses TTG had typical ATN start codons, and terminated with TAA/TAG or a single T residue. In addition, UUA (Leu2), UCU (Ser2), CGA (Arg), and GGA (Gly) were the four most frequently used codons. All tRNAs were folded into a secondary cloverleaf structure, except for $tRNA^{Ser}$, which lacks the DHU arm. The analysis of the nonsynonymous and synonymous substitution rates of PCGs showed that a strong purifying and negative selection exists in those buprestid beetles. Phylogenetic analyses within the subfamily Agrilinae were performed using concatenation methods based on multiple matrices from mitochondrial genes. The phylogenetic results indicated that *A. zanthoxylumi* is clustered with other species of *Agrilus*. All phylogenetic trees supported the monophyly of Agrilinae and Trachini, but the tribal relationship in Agrilinae remains ambiguous.

Keywords: *Agrilus zanthoxylumi*, mitogenome, next-generation, phylogeny, jewel beetle.

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INTRODUCTION

The family Buprestidae is one of the largest in the order Coleoptera, containing six subfamilies, 521 genera, and over 15,000 species (Jendek, 2024). The Agrilinae is the largest subfamily in the family Buprestidae and indeed within the class Insecta, according to modern classification systems; it contains four tribes (Coraebini, Agrilini, Aphanisticini, and Tracheini) (Bellamy, 2008; Huang, Wei, Lu, & Shi, 2023). Larvae of species in the tribes of Agrilini and Coraebini are wood-boring species (Jendek, 2016; Jendek & Nakla´dal, 2019; Kelnarova, Jendek, Grebennikov, & Bocak, 2019), while larvae of the tribes of Aphanisticini and Tracheini are leaf miners (Kato & Kawakita 2023; Mahesh, Chandran, Manjunatha, & Balan, 2013; Shi, Wu, Dai, Xu & Song, 2023). The genus *Agrilus* comprises over 3,000 species, which are distributed globally (Bozorov, Luo, Li, & Zhang, 2019). Some species are important pests in forest ecosystems causing economic problems for forest managers (Herms & McCullough, 2014). For example, *Agrilus planipennis*, known as the emerald ash borer, is a pest of ash trees (*Fraxinus* spp.) (Jendek & Poláková, 2014), while *Agrilus auroguttatus*, the gold-spotted oak borer, poses a threat to oak trees (Lopez, Rugman-Jones, Coleman, Hoddle, & Stouthamer, 2014). Currently, the number of fully sequenced mitochondrial genomes for the *Agrilus* recorded in the NCBI database is fewer than 10, which constitutes less than 1% of the species within this genus. No research on the mitochondrial genome of *Agrilus zanthoxylumi* has been conducted or completed previously.

Despite extensive taxonomic research based on morphological characters, the phylogenetic relationships within the subfamilies and tribes of the Buprestidae remain uncertain (Cobos, 1980, 1986; Tôyama, 1987; Hoghoyoski, 2009; Huang, Chen, Wei, & Shi, 2022). Wei, Huang, & Shi (2023) highlighted the ongoing debate over the phylogenetic position of the genus *Sambus*. Evans, Mckenna, Bellamy, & Farrell (2015) constructed the first comprehensive phylogenetic trees (141 species) using nuclear and mitochondrial data, confirming the monophyly of the family Schizopodidae and certain subfamilies of the family Buprestidae. Molecular studies suggest *Agrilus viridis* is monophyletic, with feeding forms indicating distinct species (Bernhard, Fritzsch, Glöckner, & Wurst, 2005; Pentinsaari, Mutanen, & Kaila, 2014; Pellegrino, Curletti, Liberatore, & Cucco, 2017). In addition, DNA barcoding was found effective for the identification of *Agrilus* species by Kelnarova et al (2019). Recently, Wei (2022) confirmed the monophyly of the Agrilinae but found the Coraebini to be paraphyletic in a phylogenetic analysis of four subfamilies and 15 species of the Buprestidae.

In the present study, the mitogenome of *A. zanthoxylumi* Li, 1989 (花椒窄吉丁) was assembled for the first time, and was used to conduct a comprehensive phylogenetic analysis within the subfamily Agrilinae combining the published agriline mitogenome data in the NCBI database.

MATERIALS AND METHODS

Sample collection, identification, and DNA extraction

The adult specimens of *A. zanthoxylumi* used in this study were collected on 26 March 2022 from Zhenfeng County, Guizhou Province, China (25°24′N, 105°35′E). Specimens were immediately immersed in 100% ethanol after collected and stored at −20°C until used for DNA extraction. A voucher specimen has been retained at the Institute of Entomology, Guizhou University, Guiyang, Guizhou, China (GUGC). One specimen was sent to Berry Genomics Co., Ltd. (Beijing, China) for DNA extraction and sequencing. The DNA concentrations were determined using a Qubit® DNA Assay Kit and a Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). For sample preparation for sequencing, 1.5 μg of DNA was used as the input material for each sample. A TruSeq Nano DNA HT sample preparation kit (Illumina, San Diego, CA, USA) was used to construct a sequencing library for each sample following the manufacturer's instructions. An index code was inserted into the attribute sequence of each sample.

Mitogenome sequencing, assembly, and annotation

Illumina TruSeq libraries with an average insert size of 350 bp were generated and sequenced on an Illumina NovaSeq 6000 platform (Beijing Berry Bioinformatics Technology Co., Ltd., Beijing, China), yielding 150-bp paired-end reads. An initial annotation of the mitogenome was conducted using Mitoz 2.4-alpha software (Meng, Li, Yang, & Liu, 2019) with the available invertebrate mitochondrial genetic information. The locations and secondary structures of transfer RNA genes (tRNAs) were reconfirmed and predicted using the MITOS web server (http://mitos2.bioinf.uni-leipzig.de/index.py; accessed on September 9, 2022) (Bernt et al., 2013) and the tRNAscan-SE search server (http://lowelab.ucsc.edu/tRNAscan-SE; accessed on September 20, 2022) (Lowe & Chan, 2016). Next, tRNA secondary structures were manually drawn in Adobe Illustrator. Circular mitogenome maps were visualized using Geneious Prime bioinformatics software (Kearse et al., 2012).

Thirteen protein-coding genes (PCGs) present in the mitogenome of *A. zanthoxylumi* were manually examined and aligned with published mitogenome sequences of other species from the Agrilinae, including *Agrilus ornatus* (NC_064400), *A. planipennis* (NC_030758), *Coraebus diminutus* (NC_064326), and *Meliboeus sinae* (NC_064327).

Sequence analysis

The relative synonymous codon usage score was calculated using MEGA 7 software (Kumar, Stecher, & Tamura, 2016), and base skew values were determined using the following formulas: AT skew = (A − T)/(A + T); and GC skew = (G − C)/(G + C) (Perna & Kocher, 1995; Hassanin, Léger, & Deutsch, 2005). Geneious Prime was used to assess the nucleotide composition of the entire mitogenome, PCGs, ribosomal RNA genes (rRNAs), tRNAs, and AT content (Kearse et al., 2012). Geneious Prime was also used to produce circular mitogenome maps. Tandem repeats were

identified using the online Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.html; accessed on September 13, 2022) (Benson, 1999). The complete mitogenome of *A. zanthoxylumi* was successfully obtained and uploaded to GenBank under accession number NC_081980. Next, the nonsynonymous substitution rate (Ka) and synonymous substitution rate (Ks) of 13 PCGs were calculated from three *Agrilus* species (i.e., *A. zanthoxylumi, A. planipennis*, and *A. ornatus*) using DnaSP version 5 (Librado & Rozas, 2009).

Phylogenetic analysis

A comprehensive phylogeny inference was performed using the mitogenome sequences of 18 agriline beetle species (ingroup) and 4 other buprestid beetle species (outgroup), which were downloaded from the NCBI database, although the newly sequenced species, *A. zanthoxylumi*, was not included (Table 1).

Table 1. List of species analyzed in this study and corresponding GenBank accession IDs.

Family	Subfamily	Tribe	Species	Length	ID	Reference
Buprestidae	Agrilinae		*Sambus femoralis*	15,367	NC_064328	Wei 2022
			Sambus kanssuensis	15,411	NC_080317	Huang et al. 2023
		Tracheini	*Trachys variolaris*	16,771	NC_060322	Cao and Wang 2019a
			Trachys auricollis	16,429	NC_046045	Xiao, Zhang, Long, Guo, Xu, Dai & Wang, 2019
			Habroloma sp.	16,273	OQ784266	Unpublished
		Coraebini	*Coraebus diminutus*	15,499	NC_064326	Wei 2022
			Coraebus cloueti	15,514	NC_064325	Wei 2022
			Coraebus cavifrons	15,686	NC_060321	Cao and Wang 2019b
			Meliboeus sinae	16,108	NC_064327	Holy'nski 1993
		Agrilini	*Agrilus adelphinus*	15,732	NC_071932	Unpublished
			Agrilus discalis	15,784	NC_069980	Huang et al. 2023
			Agrilus mali	16,204	MN894890	Sun et al. 2020
			Agrilus ornatus	15,789	NC_064400	Unpublished
			Agrilus planipennis	15,942	NC_030758	Duan et al. 2017
			Agrilus sichuanus	16,521	NC_064324	Wei 2022
			Agrilus zanthoxylumi	16,320	NC_081980	This study
		Aphanisticini	*Cantonius szechuanensis*	15972	NC_080316	Huang et al. 2023
			Endelus continentalis	16,246	NC_067866	Huang et al. 2023
	Julodinae		*Julodis variolaris* (outgroup)	16,227	NC_071967	Wei et al. 2023
	Polycestinae		*Coomaniella copipes* (outgroup)	16,196	NC_063146	Huang et al. 2022
			Ptosima chinensis (outgroup)	16,115	NC_071966	Wei et al. 2023
Dryopidae			*Dryops ernesti* (outgroup)	15,672	NC_036275	Linard et al. 2016

The nucleotide sequences of 13 PCGs and 2 ribosomal RNAs (12S rRNA + 16S rRNA), as well as the amino acids from the 13 PCGs, were aligned using the highly accurate L-INS-I strategy in MAFFT v7.394 software (Katoh & Standley, 2013). The aligned sequences were then trimmed using the heuristic "automated1" method in trimAl tool v1.4.1 (Capella-Gutiérrez, Silla-Martínez, & Gabaldón, 2009) to remove positions containing only gaps or ambiguities. Finally, the trimmed sequences were

concatenated using FASconCAT-G v1.04 software (Kück & Longo, 2014). Finally, four matrices were generated for the phylogeny inference: (1) the amino acid sequences of the 13 protein-coding genes (PCGs_faa); (2) the nucleotide sequences of 13 PCGs, excluding the third codon position (PCG12_fna); (3) the nucleotide sequences of 13 PCGs plus two ribosomal RNA sequences (PCG_rrna); and (4) PCG12_fna plus two rRNAs with the two ribosomal RNA sequences (PCG12_rrna). The third codon positions were excluded from the nucleotide-based analyses to reduce the potential bias or long-branch attraction issues caused by substitution saturation among species belonging to different genera (Leebens-Mack et al., 2005; Stefanović, Rice, & Palmer, 2004). The models used to construct phylogenetic trees for different matrices are shown in Table 2. In this study, values greater than 98% (SH-aLRT, UFBoot2) and 0.99% (posterior probability) are considered to be of a "high" support; values of 80% to 98% for SH-aLRT, 95% to 98% for UFBoot2, and 0.95% to 0.99% for the posterior probability are considered to be of a "moderate" support; and values of 95% for UFBoot2 and 0.95% for the posterior probability are considered to be of a "low" support. Phylogenetic trees were visualized using FigTree 1.4.3 software (available at http://tree.bio.ed.ac.uk/software/figtree).

Table 2. Best-fit models of four datasets used for phylogeny.

Matrices	Maximum likelihood	Bayesian inference
PCGs_faa	LG+I+G; mtART+H4; mtART+C20+F+G	CAT+GTR
PCG12_fna	GTR+I+G	
PCG_rrna	GTR+F+I+G4	CAT+GTR
PCG12_rrna	GTR+I+G	

RESULTS AND DISCUSSION

Mitogenome structure and organization

The complete mitochondrial genome of *A. zanthoxylumi* (GenBank accession no. NC_081980) is 16,320 bp in length and includes 13 PCGs, 22 tRNA genes, 2 rRNA genes, and an AT-rich region. The length and composition of the mitochondrial genes of this species are highly similar to those of other buprestid species (Duan et al., 2017; Cao & Wang, 2019a, 2019b; Chen, Wei, & Shi, 2021; Peng, Liu, Wang, & Zhan, 2021; Wei, 2022). As in most Buprestidae, 23 of the 37 genes are located on the major strand (F-strand), while the remaining 14 genes (including 8 tRNAs, 2 rRNAs, and 4 PCGs) are encoded on the minor strand (R-strand) (Fig. 1; Table 3). We also identified 13 gene overlaps (−40 to −1 bp in length) and 10 intergenic spacers (1-58 bp in length) within the mitogenome of *A. zanthoxylumi* (Table 3). Finally, we discovered a 7-bp overlapping region between ATP8 and ATP6 and between ND4 and ND4L; these small overlaps have often been reported in the mitochondrial genomes of insects (Huang et al., 2022).

Table 3. Organization of the *Agrilus zanthoxylumi* mitochondrial genome.

Gene	Direction	Location (bp)	Size (bp)	Codon		Anti-codon	IGN
				Start	Stop		
$tRNA^{Ile}$	F	1-63	63	-	-	GAT	
$tRNA^{Gln}$	R	61-129	69	-	-	TTG	-3
$tRNA^{Met}$	F	132-200	69	-	-	CAT	2
ND2	F	201-1226	1026	ATA	TAA	-	0
$tRNA^{Trp}$	F	1226-1295	70	-	-	TCA	-1
$tRNA^{Cys}$	R	1354-1415	62	-	-	GCA	58
$tRNA^{Tyr}$	R	1416-1477	62	-	-	GTA	0
COX1	F	1488-3024	1537	ATG	T-	-	10
$tRNA^{Leu}$	F	3025-3092	68	-	-	TAA	0
COX2	F	3093-3774	682	ATT	T-	-	0
$tRNA^{Lys}$	F	3775-3844	70	-	-	CTT	0
$tRNA^{Asp}$	F	3845-3908	64	-	-	GTC	0
ATP8	F	3909-4064	156	ATC	TAG	-	0
ATP6	F	4058-4732	675	ATG	TAA	-	-7
COX3	F	4732-5520	789	ATG	TAG	-	-1
$tRNA^{Gly}$	F	5527-5590	64	-	-	TCC	6
ND3	F	5591-5944	354	ATT	TAG	-	0
$tRNA^{Ala}$	F	5948-6012	65	-	-	TGC	3
$tRNA^{Arg}$	F	6012-6075	64	-	-	TCG	-1
$tRNA^{Asn}$	F	6076-6140	66	-	-	GTT	-1
$tRNA^{Ser}$	F	6141-6207	67	-	-	TCT	0
$tRNA^{Glu}$	F	6212-6275	64	-	-	TTC	4
$tRNA^{Phe}$	R	6275-6337	63	-	-	GAA	-1
ND5	R	6338-8060	1723	ATA	T-	-	0
$tRNA^{His}$	R	8061-8125	65	-	-	GTG	0
ND4	R	8126-9461	1336	ATG	T-	-	0
ND4L	R	9455-9739	285	ATG	TAA	-	-7
$tRNA^{Thr}$	F	9755-9817	63	-	-	TGT	15
$tRNA^{Pro}$	R	9817-9881	65	-	-	TGG	-1
ND6	F	9883-10,389	507	ATT	TAA	-	1
CYTB	F	10,389-11,537	1149	ATG	TAG	-	-1
$tRNA^{Ser}$	F	11,536-11,602	67	-	-	TGA	-2
ND1	R	11,623-12,573	951	TTG	TAA	-	20
$tRNA^{Leu}$	R	12,575-12,642	68	-	-	TAG	1
16S rRNA	R	12,603-13,931	1329	-	-	-	-40
$tRNA^{Val}$	R	13,914-13,982	69	-	-	TAC	-18
12S rRNA	R	13,983-14,794	812	-	-	-	0
A+T rich region	F	14,795-16,320	1526	-	-	-	0

The overall base composition of the complete mitochondrial genome was determined to be as follows: adenine (A), 40.5%; thymine (T), 34.2%, guanine (G), 10.7%, and cytosine (C), 14.6%. The mitogenome as a whole was found to have a relatively high AT content (74.7%) and correspondingly low GC content. These findings were consistent with those reported by Wei (2022). As observed in other buprestid species, a slightly positive AT bias (0.084) was found for the complete mitochondrial genome (Duan et al., 2017; Xiao et al., 2019; Peng et al., 2021; Huang et al., 2022; Wei, 2022).

PCGs

The 13 PCGs found in *A. zanthoxylumi* have a total combined length of 11,136 bp. These 13 genes include 9 genes (i.e., ND2, ND3, ND6, COI, COII, COIII, ATP6, ATP8, and CYTB) located on the major strand (F-strand) and 4 genes (i.e., ND1, ND4, ND4L, and ND5) located on the minor strand (R-strand) (Table 4). All AT and GC skews of the PCGs are negative, except the GC skew at the first codon position is positive (Table 4). All protein-coding sequences originated with a typical ATN codon, except for ND1, which had a TTG start codon (Table 3) (Ma, Liu, Yang, & Kang, 2009). According to mitochondrial data sourced from the NCBI database, specific modifications to the ND1 start codon are regularly found in most Buprestidae species. However, ATN is the start codon in *Coraebus cavifrons* (Cao & Wang, 2019a) and *A. planipennis* (Duan et al., 2017). Furthermore, nine PCGs were found that possessed complete stop codons (i.e., TAA/TAG), whereas four (i.e., COI, COII, ND4, and ND5) had incomplete stop codons (i.e., T-).

Table 4. Nucleotide composition, AT skew, and GC skew of different regions of the mitochondrial genome of *Agrilus zanthoxylumi*.

Genes	Size (bp)	Nucleotides composition					ATskew	GCskew
		T (%)	C (%)	A (%)	G (%)	A+T (%)		
Complete mitogenome	16,320	34.2	14.6	40.5	10.7	74.7	0.084	−0.154
Protein-coding genes	11,136	41.8	13.3	31.9	13.0	73.7	−0.134	−0.011
1st codon position	3713	38.4	10.6	36.7	14.2	75.1	−0.023	0.145
2nd codon position	3711	42.9	17.0	24.9	15.2	67.8	−0.265	−0.056
3rd codon position	3712	44.2	12.2	34.0	9.6	78.2	−0.130	−0.119
tRNA	1447	36.7	12.6	40.6	10.2	77.3	0.050	−0.105
rRNA	2141	34.6	14.2	43.2	8.0	77.8	0.111	−0.279
16S rRNA	1329	34.8	13.8	43.7	7.7	78.5	0.113	−0.284
12S rRNA	812	34.4	14.7	42.4	8.6	76.8	0.104	−0.262
A+T rich region	1526	34.2	16.6	41.2	7.9	75.4	0.093	−0.355

Subsequently, the nonsynonymous substitution rate (Ka), the synonymous substitution rate (Ks), and the Ka/Ks ratio (ω) were calculated for each PCG of the three species from the genus *Agrilus* (Fig. 2). The value of COXI was obviously lower than others, which indicates that the COXI gene has a relatively slower evolutionary rate; this phenomenon is almost universal at all insects (Liu et al., 2012; Li, Hu, & Hua, 2019; Gong et al., 2018). Meanwhile, COX3 showed significantly higher ω values than the other PCGs (Fig. 2). Notably, the ω values of all PCGs were from 0.102 to 0.849 ($0<\omega<1$), suggesting the presence of purifying selection at these loci. Therefore, all mitochondrial PCGs were used to analyze the phylogenetic relationships among Buprestidae species.

Finally, the relative synonymous codon usage values were determined for the mitochondrial PCGs of *A. zanthoxylumi*. The four codons that were most frequently used were: UUA (L), UCU (S), CGA (R), and GGA (G) (Table 5; Fig. 3).

Table 5. Codon and relative synonymous codon usage of 13 protein-coding genes of the mitochondrial genome of *Agrilus zanthoxylumi*. An asterisk (*) represent termination codon.

Amino acid	Codon	Count	RSCU	Amino acid	Codon	Count	RSCU
Phe	UUU	319	1.74	Tyr	UAU	118	1.64
	UUC	48	0.26		UAC	26	0.36
Leu2	UUA	390	4.11	His	CAU	50	1.47
	UUG	37	0.39		CAC	18	0.53
Leu1	CUU	66	0.70	Gln	CAA	58	1.63
	CUC	7	0.07		CAG	13	0.37
	CUA	61	0.64	Asn	AAU	162	1.71
	CUG	8	0.08		AAC	27	0.29
Ile	AUU	329	1.78	Lys	AAA	88	1.59
	AUC	40	0.22		AAG	23	0.41
Met	AUA	237	1.78	Asp	GAU	53	1.63
	AUG	30	0.22		GAC	12	0.37
Val	GUU	72	1.64	Glu	GAA	65	1.60
	GUC	5	0.11		GAG	16	0.40
	GUA	87	1.98	Cys	UGU	35	1.79
	GUG	12	0.27		UGC	4	0.21
Ser2	UCU	100	2.26	Trp	UGA	84	1.71
	UCC	24	0.54		UGG	14	0.29
	UCA	93	2.10	Arg	CGU	15	1.13
	UCG	9	0.20		CGC	8	0.60
Pro	CCU	61	1.85		CGA	29	2.19
	CCC	21	0.64		CGG	1	0.08
	CCA	42	1.27	Ser1	AGU	35	0.79
	CCG	8	0.24		AGC	6	0.14
Thr	ACU	84	1.71		AGA	70	1.58
	ACC	25	0.51		AGG	17	0.38
	ACA	85	1.73	Gly	GGU	41	0.78
	ACG	2	0.04		GGC	10	0.19
Ala	GCU	67	1.74		GGA	114	2.18
	GCC	24	0.62		GGG	44	0.84
	GCA	57	1.48	*	UAA	0	0
	GCG	6	0.16		UAG	0	0

Note: RSCU, relative synonymous codon usage.

Transfer RNAs and ribosomal RNAs

The total length of all tRNAs in *A. zanthoxylumi* is 1,447 bp, with individual tRNA genes ranging in size from 62 bp (i.e., $tRNA^{Cys}$ and $tRNA^{Tyr}$) to 70 bp (i.e., $tRNA^{Trp}$ and $tRNA^{Lys}$). Eight tRNAs were encoded on the minor strand and 14 on the major strand (Table 3). The AT content (77.3%) of tRNA-associated areas was much greater than the GC content (23.7%). The AT skew of these areas was mildly positive (0.050) and the GC skew was negative (−0.105), thereby indicating a minor bias toward A and

a stronger aversion against C. In addition, the secondary structure of most tRNAs conforms to the typical cloverleaf secondary structure. However, $tRNA^{Ser}$ was an exception: it contains an unusual dihydropurine arm that forms a simple loop; this has also been observed in other buprestids (Fig. 4) (Xiao, Jia, Murphy, & Huang, 2011; Yu & Liang, 2018; Wei, 2022).

The 16S rRNA and 12S rRNA genes of *A. zanthoxylumi* are 1,329 bp and 812 bp in length, respectively (Table 3). The two rRNA genes are frequently separated by $tRNA^{Val}$ in many Buprestidae species (Cao & Wang, 2019a; Huang et al., 2022; Wei, 2022). Furthermore, the AT content of rRNA-associated regions was 77.8% and showed both a positive AT skew (0.111) and a negative GC skew (−0.279) (Table 4).

AT-rich region

The AT-rich region, also known as a control region (CR), regulates the transcription and replication of mitochondrial DNA (mtDNA) (Zhang & Hewitt ,1997; Boore, 1999; Cameron, 2014). In *A. zanthoxylumi*, the CR is located between the 12S rRNA and $tRNA^{Ile}$ genes (Fig. 1; Table 3). The CR is 1,526 bp long, and has an A+T content of 75.4%. The AT-skew and G-skew in the control area are 0.093 and −0.355, respectively.

Phylogenetic analyses

The phylogenetic analyses of the present study included 18 species from Agrilinae of the Buprestidae and four outgroup species (Table 1). Three phylogenetic trees are shown in Figs. 5, 6, and 7; other phylogenetic trees are displayed in Supplementary Figures S1-S5.

The findings from both the Maximum Likelihood (ML) and the Bayesian Inference (BI) trees consistently showed that all species within the genus *Agrilus* were clustered together. All phylogenetic trees constructed using the GTR model consistently indicated that *Endelus continentalis* and *Cantonius szechuanensis* were clustered together, while *Sambus* and *Agrilus* did not form a sister group (Fig. 7; Figs S2-S4). For the phylogenetic tree built using the PCGs_faa dataset, the topologies of the LG+I+G, mtART+H4, and mtART+C20+F+G models were consistent, as opposed to that of the CAT+GTR model. In the CAT+GTR model, the taxa *Coraebus* and *M. sinae* do not cluster together, and *E. continentalis* and *C. szechuanensis* also do not form a clade, which suggests that the Coraebini and Aphanisticini tribes are non-monophyletic. The genera *Sambus* and *Agrilus* exhibited a sister group relationship with strong node support (SH-aLRT/UFBoot =98.4/93) in the phylogenetic tree of the mtART+C20+F+G model (Fig. 5), but showed weak node support (mtART+H4 model: SH-aLRT/UFBoot =97.5/53; LG+I+G model: SH-aLRT/UFBoot =95.8/57) in the other two models (Figs. S1 and S2). Other phylogenetic trees indicate that *Sambus* and *Agrilus* do not cluster together. In the past several decades, Kubáň, Majer, & Kolibáč (2000) transferred *Sambus* from the Coraebini to Agrilini based on the oviposition behavior. Later, the genus *Sambus* was placed in *incertae sedis* (Kubáň, 2016). The research results of many researchers indicate that the phylogenetic position of *Sambus* is still controversial (Huang et al., 2022; Huang et al., 2023; Wei et al., 2023). In the

phylogenetic tree based on the PCG_rrna, *S. femoralis* is positioned at the base of the Agrilinae subfamily, indicating a distant relationship with *Agrilus*, while Coraebini remains a non-monophyletic group (Figs. S4 and S5).

CONCLUSION

In summary, the complete mitogenome of *A. zanthoxylumi*, an important invasive pest of Chinese prickly ash (*Zanthoxylum bungeanum* Maxim.) in China, was successfully sequenced and assembled. Further detailed analyses of the *A. zanthoxylumi* mitogenome is provided, detailing gene structures, skew values, and codon usages in different regions, including rRNAs, tRNAs, PCGs, and the AT-rich region. The phylogenetic results supported that the subfamily Agrilinae and the tribe Trachini are monophyletic groups, but the Coraebini and Aphanisticini are non-monophyletic groups. Our study provide new data which can support future phylogenetic studies of the Agrilinae and improves our understanding of their mitogenomic and taxonomic characteristics. However, the phylogenetic analysis of the present study did not resolve the taxonomic status of some species; therefore, additional mitogenome sampling will be needed to resolve the molecular phylogeny of the Agrilinae to better understand the phylogenetics of the Agrilinae subfamily.

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DATA RESOURCES

The genome sequence data are available in GenBank (https://www.ncbi.nlm.nih.gov/) under accession no. NC_081980.

CONFLICTS OF INTEREST

No potential conflicts of interest were reported by the author(s).

AUTHOR CONTRIBUTIONS

Jingjing Li: Conceptualization (lead); software (lead); methodology (equal); writing-original draft (lead). Bin Yan: Conceptualization (equal); methodology (equal); writing-review & editing (lead). Haitian Song: writing-review & editing (lead). Maofa Yang: Conceptualization (equal); project administration (lead); supervision (lead); writing-review & editing (lead).

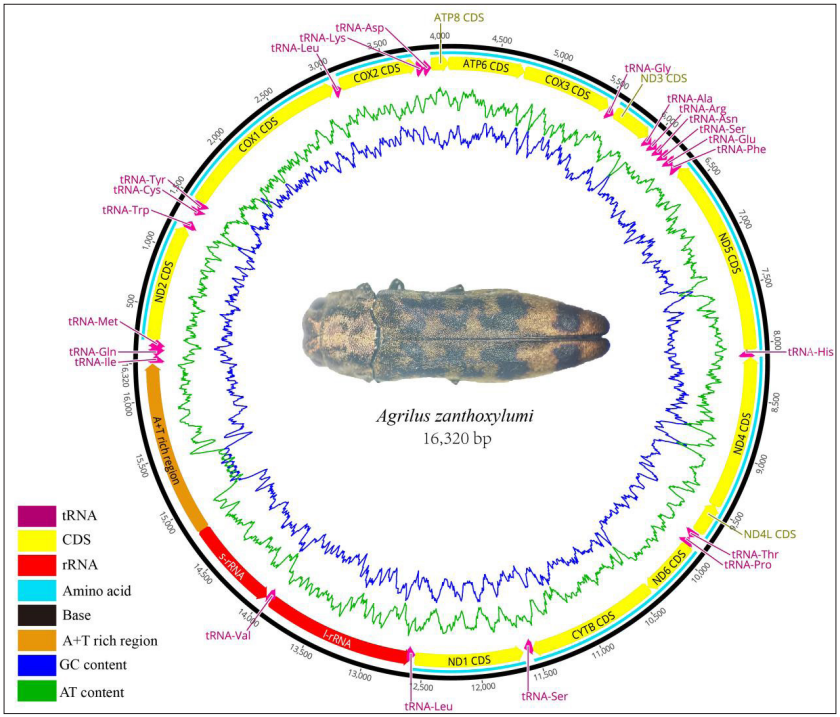

Figure 1. Circular map of the mitochondrial genome of *Agrilus zanthoxylumi*.

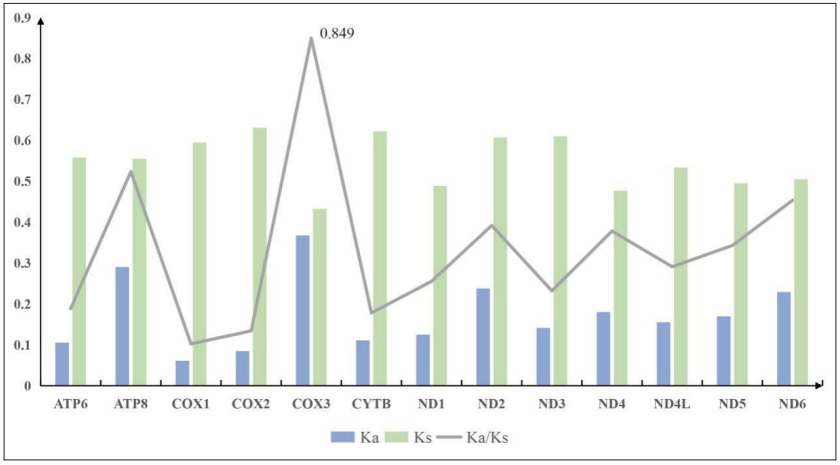

Figure 2. The nonsynonymous substitution rate (Ka), synonymous substitution rate (Ks), and Ka/Ks ratio for 13 protein-coding genes from the mitogenomes of three species of the genus *Agrilus*.

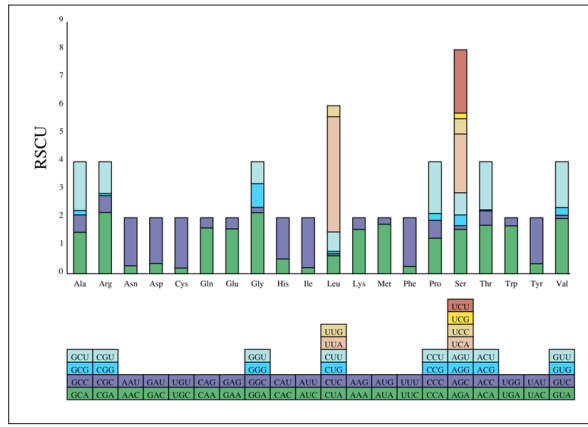

Figure 3. Relative synonymous codon usage of the mitogenome of *Agrilus zanthoxylumi*.

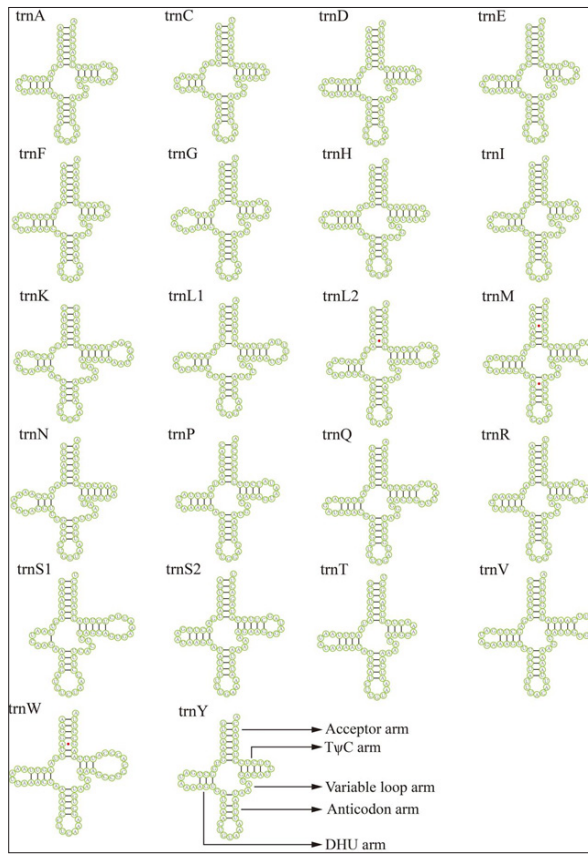

Figure 4. Secondary structures of 22 tRNAs from the mitogenome of *Agrilus zanthoxylumi*. Lines (-) indicate Watson-Crick base pairings, and dots (•) indicate unmatched base pairings.

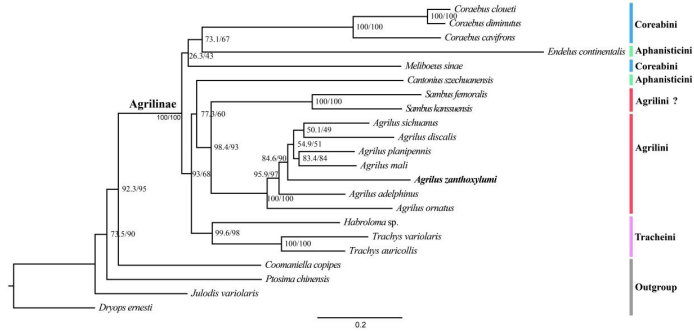

Figure 5. Phylogenetic tree based on the maximum likelihood analysis of the PCGs_faa matrix with the mtART+C20+F+G model. Note: The phylogenetic position of *Agrilus zanthoxylumi* sequenced in this study is indicated in bold. The numbers on the nodes are the SH-aLRT support (%) and ultrafast bootstrap support (%). Different branches of the tribes and outgroup are represented by different colors.

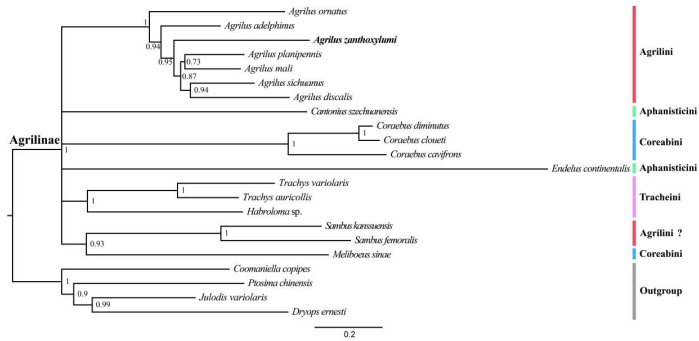

Figure 6. Phylogenetic tree based on the bayesian inference analysis of the PCGs_faa matrix with the CAT+GTR model. Note: The phylogenetic position of *Agrilus zanthoxylumi* sequenced in this study is indicated in bold. The numbers on the nodes are the SH-aLRT support (%) and ultrafast bootstrap support (%). Different branches of the tribes and outgroup are represented by different colors.

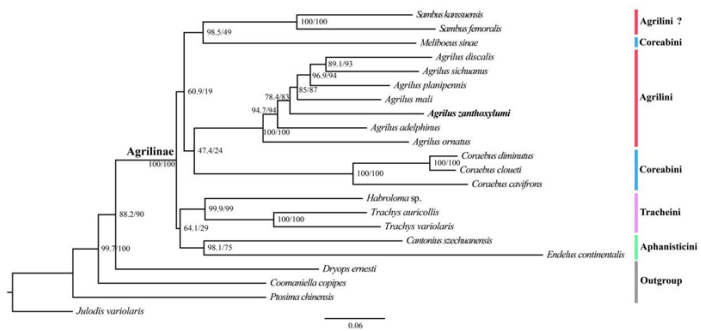

Figure 7. Phylogenetic tree based on the maximum likelihood analysis of the PCG12_rrna matrix with the GTR+I+G model. Note: The phylogenetic position of *Agrilus zanthoxylumi* sequenced in this study is indicated in bold. The numbers on the nodes are the SH-aLRT support (%) and ultrafast bootstrap support (%). Different branches of the tribes and outgroup are represented by different colors.

SUPPORTING INFORMATION

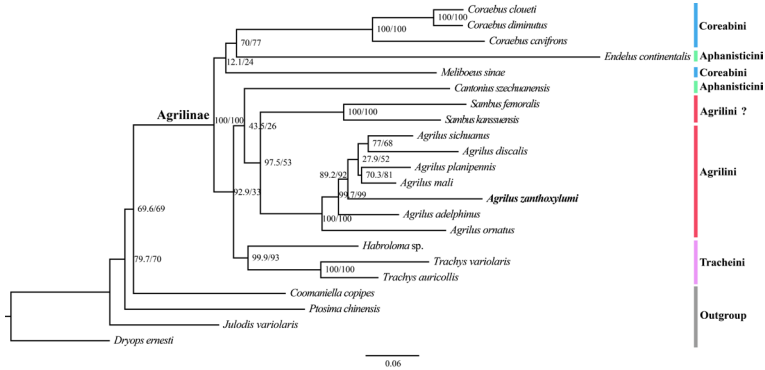

Figure S1. Pylogenetic tree based on the maximum likelihood analysis of the PCGs_faa matrix with the mtART+H4 model.

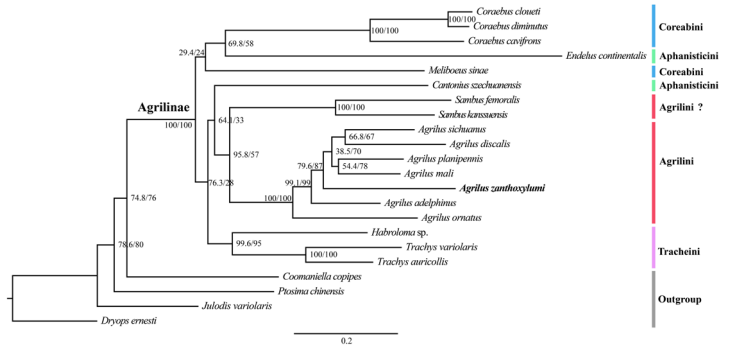

Figure S2. Pylogenetic tree based on the maximum likelihood analysis of the PCGs_faa matrix with the LG+I+G model.

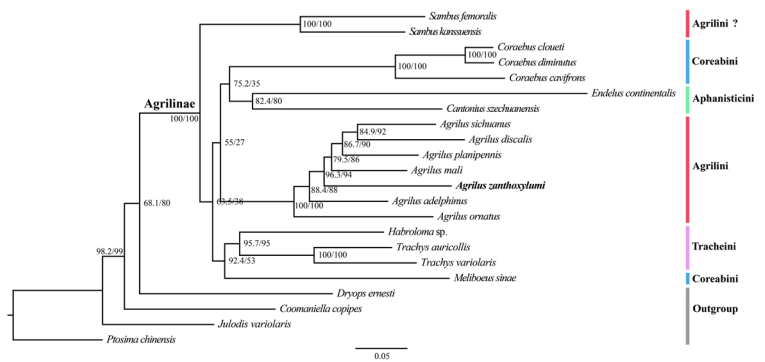

Figure S3. Pylogenetic tree based on the maximum likelihood analysis of the PCG12_fna matrix with the GTR+I+G model.

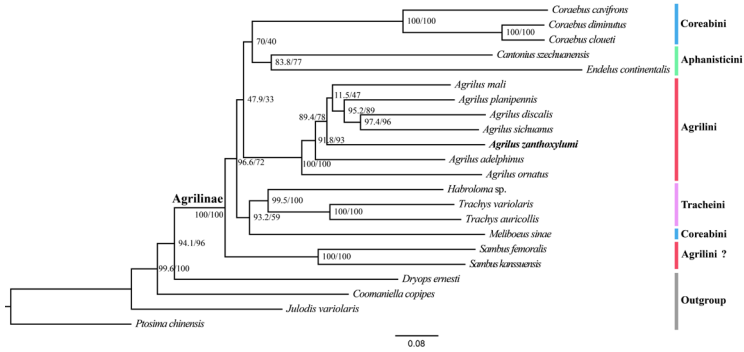

Figure S4. Pylogenetic tree based on the maximum likelihood analysis of the PCG_rrna matrix with the GTR+F+I+G4 model.

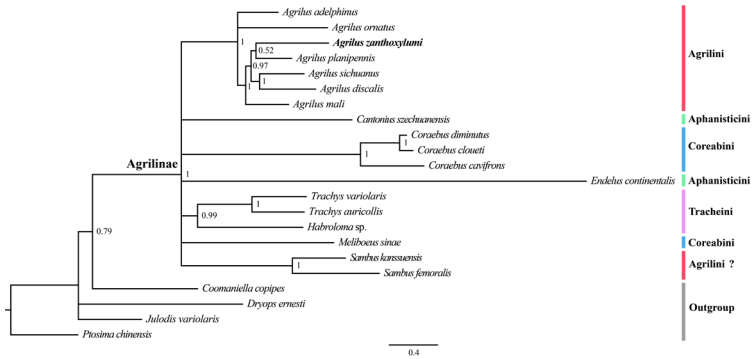

Figure S5. Pylogenetic tree based on the Bayesian inference analysis of the PCG_rrna matrix with the CAT+GTR model.

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