

Comparative Mitgenome Analysis of *Anoplophora horsfieldi* and Other Chrysomeloidea, Cucujiformia Insects Reveals Conserved Mitogenome Organization and Phylogeny

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ABSTRACT

Mitochondrial genomes are important markers using to reconstruct phylogenetic status and reveal insect molecular evolution. In this study, the mitochondrial genome (mitogenome) of *Anoplophora horsfieldi* (Coleoptera: Chrysomelidae) was determined using high-throughput sequencing. The size of circular mitogenome is 15,796 bp and it includes a typical structure of 13 protein-coding genes (PCGs), two ribosomal RNA genes (*rRNAs*), 22 transfer RNA genes (*tRNAs*) and an adenine + thymine (A+T)-rich region. The base composition of the major strand is A: 39.74%, T: 39.57%, G: 8.22%, and C: 12.47%, with a A+T content of 79.31%. The genomic analyses indicated that its gene arrangement and content is similar to that of other Cucujiformia species. The *Anoplophora* species control region sequence with rich A+T content exhibited high genetic variability. All PCGs initiate with ATN and terminate with the TAA or TAG except for COXI-COXIII and ND3-ND5, where they end with an incomplete stop codon (T-). All tRNAs form clover-leaf structure only apart from trnS (AGN) which possess a reduced DHU arm. The motifs 'ATGATAA' between ND4L and ND4, was more conserved than that between trnS (UCN) and ND1 and between ATP8 and ATP6 in the mitogenomes of Cucujiformia. The 1,143 bp A+T-rich area includes a 16 bp poly-T stretch, 14 bp poly-A stretch region, three microsatellite-like repeats of (TA)_n and three other random repetitive sequences. Based on 13 PCGs of 118 Cucujiformia mitogenomes, the phylogenetic analyses are reconstructed with both maximum likelihood and Bayesian analyses and a consistent topology is formed. The results show that *A. horsfieldi* grouped with *A. glabripennis* and *A. chinensis* with high nodal supports. It also supports such phylogenetic relationships of ((Lymexyloidea + Tenebrionoidea) + (Curculionoidea + (Chrysomeloidea + (Cucujoidea + (Cleroidea + Coccinelloidea)))) within Cucujiformia. Therefore, *A. horsfieldi* mitogenome enriches our understanding of the phylogenetic relationship of Chrysomelidae. In addition, it is used to establish phylogenetic trees and further study the phylogenetic relationship between Cucujiformia and Chrysomeloidea.

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HQ investigation; collect samples, analyze the data. YL investigation; collect samples; contribute analysis tools. YZ investigation; collect samples; contribute analysis tools; organize tables and beautify pictures. JL analyze the data; prepare figures and tables. ZZ investigation, collect samples. LJ conceive and designed the experiments; write the paper.

Key words

Anoplophora horsfieldi, Coleoptera, Cucujiformia, Chrysomeloidea, mitochondrial genome, molecular phylogeny

INTRODUCTION

Anoplophora is an important genus in the longhorned beetle family Cerambycidae. They are widely distributed in the Oriental and eastern Palearctic regions,

and contain 36 species (McDougall, 2001; Richard and Gregory, 2002). Several notorious pest insects belong to this genus (McDougall, 2001). *Anoplophora horsfieldi* has body length of about 35 mm. *Camellia sinensis*, *Celtis sinensis*, *Quercus glauca* and *Ulmus pumila* are main host plants. Adults appeared in summer, and lived in low and middle mountainous areas. This species can be found in India, Vietnam and China (Chen *et al.*, 1959). Most of the Cerambycidae species, as a large family in the Coleoptera order, are forestry pests with above 25,000 species worldwide (Sama *et al.*, 2010; Wang *et al.*, 2012). Although Cerambycidae shows large taxonomic diversity, it is still limited knowledge about the Cerambycidae mitogenome.

Mitogenomes are important molecular markers,

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which can be used for exploring the phylogenetics, population genetics and phylogeographics of species due to low recombination rate, maternal inheritance, conserved gene organization and orientation and high mutation rate (Dowton *et al.*, 2002; Jin *et al.*, 2004; Wang and Tang, 2018). In general, the metazoan mitogenome forms a closed-circular structure, with a length of 15 to 18 kilobases (kb) (Dowton *et al.*, 2002; Lu *et al.*, 2013; Cameron, 2014), including 13 PCGs, 22 tRNAs, 2 rRNAs and a non-coding region (A+T-rich region) that in other Cucujiformia and in invertebrates is named as A+T-rich region because of its extremely high content in Adenines and Thymines (Wolstenholme, 1992; Cameron, 2014; Sun *et al.*, 2017; Zhang *et al.*, 2019). Recent high throughput sequencing development has enriched insect mitogenome datasets and accelerated the research on insect molecular evolution, too. At present, insect mitochondrial genome data has been used for species identification, comparative genomics, evolutionary genetics, reconsecration of phylogeny and phylogeography (Cameron and Whiting, 2008; Hao *et al.*, 2012; Ma *et al.*, 2012; Timmermans *et al.*, 2014; Li *et al.*, 2016).

Coleoptera is an ancient order and can adapt to a variety of habitats (Li *et al.*, 2016). Coleoptera has approximately 400,000 described species and contains Adephaga, Archostemata, Myxophaga and Polyphaga (Crowson, 1960; Nie and Yang, 2013; Zhang, 2013). Based on morphological features, Crowson (1960) suggested Polyphaga can be divided into five infraorders and 18 superfamilies. However, Bouchard *et al.* (2011) think that Polyphaga can be divided into six infraorders and 16 superfamilies. Because of different phylogenetic positioning of some superfamilies, so these classification systems contain different numbers of infraorders (Nie and Yang, 2013). Cucujiformia, as the most highly diversified infraorders of Polyphaga (Crowson, 1960; Ødegaard, 2000; Bouchard *et al.*, 2009; Wang and Tang, 2018) includes six superfamilies and most of two superfamilies species (Curculionoidea and Chrysomeloidea) are plant-feeding beetles (Grimaldi and Engel, 2005). Recently, Robertson *et al.* (2015) and Yuan *et al.* (2016) suggest that Cucujiformia can be divided into seven superfamilies (Cleroidea, Coccinelloidea, Lymexyloidea, Tenebrionoidea, Cucujoidea, Chrysomeloidea, Curculionoidea). Moreover, long-horned beetles and leaf beetles in Chrysomeloidea and weevils in Curculionoidea are considered as Coleoptera 'Phytophaga'. Chrysomeloidea contains the families Chrysomelidae, Cerambycidae, Megalopodidae, Vesperidae, Oxypeltidae, Disteniidae and Orsodacnidae (Hunt *et al.*, 2007).

The phylogeny and evolution of Chrysomeloidea have received considerable attention of systematists.

Nonetheless, the interrelationships (families and subfamilies) are unclear and many chrysomeloid evolutionary questions still persist. This is especially true for Cerambycidae, for which there is a few molecular phylogenies, however, the numbers and species used in the construction of phylogenetic trees are limited. Several studies have explored the phylogenetic relationships of Chrysomeloidea by using the combined data including morphological data (Farrell and Sequeira, 2004; Gómez-Zurita *et al.*, 2007) and molecular data (18S rRNA Hunt *et al.*, 2007; 28S rDNA Marvaldi *et al.*, 2009, and partial mitochondrial genes Bocak *et al.*, 2014; Li *et al.*, 2016; Wang and Tang, 2018). In addition, there are very few molecular studies about Chrysomeloidea taxa. Currently, some researchers have found some new evidence to understand the Chrysomeloidea phylogenetic relationships, but some conflicting results are often achieved due to the differences of datasets and analytical methods. Therefore, the phylogenetic relationships of Cucujiformia and Chrysomeloidea need to be further evaluated.

In this study, we achieved the complete sequence of the mitogenome of *A. horsfieldi* using next-generation sequencing and compared it with other cucujiform insects. We aimed to analyze the structural characteristics of *A. horsfieldi* mitogenome and compared it with previously sequenced *Anoplophora* mitogenomes. Moreover, based on all 117 mitogenome sequences also used BI and ML methods to reconstruct a tentative phylogeny to evaluate relationships among Cucujiformia and Chrysomeloidea. Our review is structured taxonomies, commencing with the phylogenetic neighborhood of Chrysomeloidea and their relatives, followed by sections on Chrysomelidae, Cerambycidae and then Cucujiformia and its relatives. The sequence characteristics and annotation of *A. horsfieldi* mitogenome will be a significant increase in further research of Cucujiformia and Chrysomeloidea mitogenome structures and phylogenetics.

MATERIALS AND METHODS

Sample collection and genomic DNA extraction

The specimen of the *A. horsfieldi*, was sampled from an adult vulture captured in the Yongxing Town, Mianyang City, Sichuan Province, China in June 2020 (104°39'5.43"E, 31°27'39.06"N, 475 m.a.s.l). The specimen was preserved in 95% ethanol and stored -76°C until genomic DNA extraction. Total genomic DNA was extracted from the thorax muscle of a single specimen using an E.Z.N.A.® Tissue DNA Kit (Omega, Norcross, GA) abiding by the manufacturer's instructions. The extracted DNA quality was examined by 0.9% agarose gel electrophoresis (w/v) and used to sequence the whole mitogenome of *A.*

horsfieldi with next-generation sequencing.

Library preparation and sequencing

Genome sequencing libraries with about 400 bp of insertion fragment were constructed with a NEXTflex™ Rapid DNA-Seq Kit (Illumina, San Diego, CA) according to the manufacture's protocols. The library was sequenced on Illumina HiSeq X Ten platform to produce 150 bp paired end reads (300 cycles).

Mitochondrial genome assembly and analysis

SOAPdenovo v2.0 (Luo *et al.*, 2012), MITObim v1.8 (Christoph *et al.*, 2013) and NOVOPlasty v2.7.1 (Dierckxsens *et al.*, 2017) were alternate application to assembly the mitochondrial genome. The assembled mitochondrial fragments were identified by BlastX using *A. chinensis* (Li *et al.*, 2015) (NC_029230) and *A. glabripennis* (Fang *et al.*, 2016) (NC_008221) mitochondrial genes as queries. Prediction and annotation of 13 PCGs, 22 tRNA and 2 rRNA genes were performed with DOGMA (<http://dogma.cccb.utexas.edu/>) or MITOS (<http://mitos.bioinf.uni-leipzig.de/index.py>) using the support of annotation from reference mitogenome.

The mitogenome sequence is further verified and verified below. 12S and 16S rRNA genes were determined by comparison using homologous sequences of mitochondrial DNA from other *Anoplophora* species with ClustalX version 2.0 (Larkin *et al.*, 2007). 13 PCG sequences were translated into putative proteins according to the Invertebrate Mitochondrial Genetic Code. The base composition of nucleotide sequences was calculated by skewness on the basis of the following formulas: AT skew = $[A-T]/[A+T]$ and GC skew = $[G-C]/[G+C]$ (Perna and Kocher, 1995). The content of A+T and relative synonymous codon usage (RSCU) values were described with MEGA 7.0 (Kumar *et al.*, 2016). The tRNA genes were resolved with the tRNAscan-SE 1.21 (<http://lowelab.ucsc.edu/tRNAscan-SE/>) (Lowe and Chan, 2016) and the MITOS Web Server (Perna and Kocher, 1995; Bernt *et al.*, 2013). The tRNAs secondary structure were achieved with RNAviz v2.0 (De Rijk *et al.*, 2003). The tandem repeats of the A+T-rich region were explored by the tandem repeat finder (<http://tandem.bu.edu/trf/trf.html>) (Benson, 1999; Timmermans *et al.*, 2014).

Phylogenetic analysis

The newly sequenced mitogenomes of *A. horsfieldi* was aligned with 116 mitogenomes of Cucujiformia and Chrysomeloidea available in GenBank, with *Prosopocoilus gracilis* (Coleoptera: Scarabaeiformia: Lucanidae: KP735805), *Cheirotonus jansonii* (Coleoptera: Scarabaeiformia: Scarabaeidae: NC_023246) and

Necrophila americana (Coleoptera: Staphyliniformia: Silphidae: NC_018352) as outgroups (Supplementary Table S1). The multiple alignments (13 PCGs concatenated nucleotide sequence datasets) were performed with ClustalX soft (v2.0 Larkin *et al.*, 2007) with the default settings. Accession numbers of all mitogenomes are listed in the phylogenetic trees (Supplementary Table S1). We employed the nucleotide sequences of the 13 PCGs as the dataset to construct the maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees. The nucleotide sequences of 13 PCGs from all 117 Cucujiformia mitogenome were aligned separately in MEGA 7.0 (Kumar *et al.*, 2016). The final alignments were refined and the conserved sequences were identified with Gblocks v0.91b (Talavera and Castresana, 2007). Geneious 8.1.6 was used to concatenate the resulting alignments (Kearse *et al.*, 2012). The optimal nucleotide substitution model was selected on the basis of the Akaike Information Criterion (Posada and Buckley, 2004) with J Model test v0.1.1 (Posada, 2008). ML and BI phylogenetic trees show different algorithms. ML phylogenetic tree was constructed by PhyML v3.0 (Guindon *et al.*, 2010) based on the best-fit model (as above) with 1,000 replicates. BI tree was conducted using MrBayes 3.2 with a GTR + I + G model, each of four chains (three hot and one cold), with run length of 20 million generations and sampling every 1,000 generations (Ronquist *et al.*, 2012). Convergence was assessed with Tracer 1.5 (Rambaut and Drummond, 2007) and trees from the first 25% of the samples were removed as burn-in. Based the value of Bayesian posterior probabilities (BPP), node support was assessed. Figtree v1.4.3 software was used to view and edit the consensus trees (Rambaut, 2009).

RESULTS AND DISCUSSION

Genome structure, organization and composition

We deposited the complete mitogenome of *A. horsfieldi* in the GenBank database (MN248534) and the mitogenome sequence of *A. horsfieldi* is 15,796 bp in size (Table I and Fig. 1), which is located in the range of whole sequenced Cucujiformia species with the length ranging from 15,064 bp in *Naupactus xanthographus* (Curculionidae) to 20,124 bp in *Ceutorhynchus obstructus* (Curculionidae) (Supplementary Table S1). Alignment results revealed 38 mitogenome regions, including 13 PCGs, two rRNA, 22 tRNA and a non-coding region with high A+T-rich composition, which is common in most animal mtDNAs (Table I). The majority strand (J-strand) of the mitogenome of *A. horsfieldi* encodes nine PCGs and fourteen tRNAs, while the remaining genes are encoded on the minority strand (N-strand) (Table I).

Table 1. The mtDNA gene arrangement of three *Anoplophora* species (*Anoplophora horsfieldi*, *Anoplophora chinensis*, *Anoplophora glabripennis*).

Gene	<i>Anoplophora horsfieldi</i> (15,796 bp)										<i>Anoplophora chinensis</i> (15,805 bp)										<i>Anoplophora glabripennis</i> (15,774 bp)									
	Position		Sizes	Anti Nucleotide	codons (tRNA)	Start	Stop*	Inter-genic nucleotide†	Position		Sizes	Anti Nucleotide	codons (tRNA)	Start	Stop*	Inter-genic nucleotide†	Position		Sizes	Anti Nucleotide	codons (tRNA)	Start	Stop*	Inter-genic nucleotide†						
	From	To							From	To							From	To												
<i>tRNA-Ile</i>	H	1	63	63	GAT		0	1	69	69	TTG		0	1	68	68	TTG							0						
<i>tRNA-Gln</i>	L	70	138	69	TTG		6	77	145	69	TTG		7	78	146	69	TTG							9						
<i>tRNA-Met</i>	H	138	207	70	CAT		-1	145	213	69	CAT		-1	146	214	69	CAT							-1						
<i>ND2</i>	H	205	1215	1011		ATA TAA	-3	214	1224	1011		ATT TAA	0	215	1225	1011		ATT TAA						0						
<i>tRNA-Trp</i>	H	1214	1278	65	TCA		-2	1223	1287	65	TCA		-2	1224	1288	65	TCA							-2						
<i>tRNA-Cys</i>	L	1271	1334	64	GCA		-8	1280	1341	62			-8	1281	1343	63	GCA							-8						
<i>tRNA-Tyr</i>	L	1335	1400	66	GTA		0	1342	1407	66	GTA		0	1344	1412	69	GTA							0						
<i>COXI</i>	H	1393	2935	1543		ATT T--	-8	1400	2942	1543		ATT T--	-8	1405	2947	1543		ATT T--						-8						
<i>tRNA-Leu</i>	H	2936	3002	67	TAA		0	2943	3007	65	TAA		0	2953	3017	65	TAA							5						
<i>COXII</i>	H	3003	3690	688		ATT T--	0	3008	3695	688		ATT T--	0	3018	3705	688		ATT T--						0						
<i>tRNA-Lys</i>	H	3691	3761	71	TTT		0	3696	3765	70	TTT		0	3706	3775	70	TTT							0						
<i>tRNA-Asp</i>	H	3749	3824	76	GTC		-13	3765	3848	84	GTC		-1	3775	3839	65	GTC							-1						
<i>ATP8</i>	H	3825	3980	156		ATT TAA	0	3849	4004	156		ATT TAA	0	3840	3995	156		ATT TAA						0						
<i>ATP6</i>	H	3974	4648	675		ATG TAA	-7	3998	4672	675		ATG TAA	-7	3989	4660	672		ATG TAA						-7						
<i>COXIII</i>	H	4648	5434	787		ATG T--	-1	4672	5460	789		ATG TAA	-1	4660	5448	789		ATG T--						-1						
<i>tRNA-Gly</i>	H	5441	5504	64	TCC		6	5464	5525	62	TCC		3	5452	5516	65								3						
<i>ND3</i>	H	5505	5856	352		ATT T--	0	5528	5879	352		ATT T--	2	5508	5856	349		ATT T--						-9						
<i>tRNA-Ala</i>	H	5857	5921	65	TGC		0	5880	5944	65	TGC		0	5857	5921	65	TGC							0						
<i>tRNA-Thr</i>	H	5919	5978	60			-3	5941	6003	63			-4	5918	5980	63								-4						
<i>tRNA-Asn</i>	H	5981	6044	64	GTT		2	6006	6069	64	GTT		2	5983	6046	64	GTT							2						
<i>tRNA-Ser</i>	H	6043	6111	69	TCT		-2	6068	6136	69			-2	6055	6123	69								8						
<i>tRNA-Glu</i>	H	6112	6174	63	TTC		0	6138	6200	63			1	6115	6177	63								-9						
<i>tRNA-Phe</i>	L	6174	6237	64	GAA		-1	6200	6263	64	GAA		-1	6177	6240	64								-1						
<i>ND5</i>	L	6241	7954	1714		ATA T--	3	6267	7977	1711		GTG T--	3	6244	7957	1714		ATA T--						3						
<i>tRNA-His</i>	L	7952	8014	63	GTG		-3	7978	8040	63			0	7957	8018	62								-1						
<i>ND4</i>	L	8015	9344	1330		ATA T--	0	8041	9370	1330		ATA T--	0	8020	9349	1330		ATA T--						1						
<i>ND4L</i>	L	9341	9628	288		ATG TAA	-4	9367	9654	288		ATG TAA	-4	9346	9633	288		ATG TAA						-4						
<i>tRNA-Thr</i>	H	9631	9697	67	TGT		2	9657	9720	64	TGT		2	9636	9699	64	TGT							2						
<i>tRNA-Pro</i>	L	9698	9762	65	TGG		0	9721	9785	65	TGG		0	9699	9772	74	GGG							-1						

Table continued on next page.....

The gene arrangement and orientation of *A. horsfieldi* mitogenome are similar to the mitogenome of typical Cerambycidae species. The differences in size of mitogenomes among Cucujiformia insects can be explained by the numbers and types of repetitive sequences in the A+T-rich regions.

Three species of the genus *Anoplophora* possess the same characteristics in the mitochondrial genome. The total base composition of *A. horsfieldi* was A (39.74%), T (39.57%), C (12.47%) and G (8.22%); A (39.48%), T (38.17%), C (13.57%) and G (8.78%) in *A. chinensis* and A (39.62%), T (38.71%), C (13.07%) and G (8.59%) in *A. glabripennis*, respectively. A heavy AT bias was found in the three species of mitochondrial genomes (79.31% in *A. horsfieldi*, 77.65% in *A. chinensis* and 78.34% in *A. glabripennis*, [Supplementary Table S2-S4](#)), which is similar to other sequenced Cerambycidae species ([Wang et al., 2013](#); [Fang et al., 2016](#); [Li et al., 2016](#)). All three *Anoplophora* species present a positive AT skew and negative GC skew in the whole mitogenome and had a higher A + T content in rRNAs than in tRNAs ([Table II](#)). In fact, the region rich in A + T has been viewed as the main source of the variations in the length of the whole mitogenome, and in the process of polynucleotide operation, insertion/deletion, the number of nucleotides is variable, and the copy numbers and types of tandem repeat elements are also very different between different species ([Zhang and Hewitt, 1997](#)).

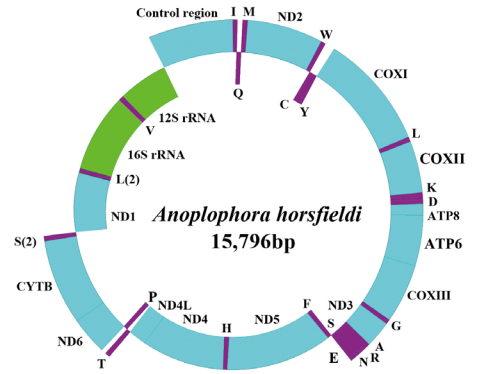


Fig. 1. Complete mitogenome organization and gene arrangement of *Anoplophora horsfieldi* from China. Genes coded on the H strand are directed to the outer ring, while the genes coded on the L strand are indicated in the interior of the ring. Genes are abbreviated as follows: ATP6 and ATP8 (subunits 6 and 8 of ATPase), COXI-COXIII (cytochrome c oxidase subunits 1-3), Cytb (cytochrome b), ND1-ND6 and ND4L (NADH dehydrogenase subunits 1-6 and 4L), 12S rRNA and 16S rRNA (ribosomal RNA of 12S and 16S), CR (control region). One-letter amino acid abbreviations were used to label the corresponding tRNA genes.

Gene	<i>Anoplophora horsfieldi</i> (15,796 bp)							<i>Anoplophora chinensis</i> (15,805 bp)							<i>Anoplophora glabripennis</i> (15,774 bp)						
	Strand	Position From To	Size Nucle- otide (bp)	Anti codons (tRNA)	Start	Stop*	Inter- genic nucle- otide†	Strand	Position From To	Size Nucle- otide (bp)	Anti codons (tRNA)	Start	Stop*	Inter- genic nucle- otide†	Strand	Position From To	Size Nucle- otide (bp)	Anti codons (tRNA)	Start	Stop*	Inter- genic nucle- otide†
ND6	H	9765 10268	504				2	H	9788 10291	504	ATT TAA	2			H	9772 10269	498	ATA TAA	-1		
Cytb	H	10268 11407	1140	ATT	TAA	-1		H	10291 11430	1140	ATG TAG	-1			H	10269 11408	1140	ATG TAG	-1		
tRNA-Ser	H	11406 11472	67	TGA		-2		H	11429 11495	67	TGA	-2			H	11407 11473	67	TGA		-2	
ND1	L	11490 12437	948		ATA TAG	17		L	11513 12460	948	ATA TAG	17			L	11491 12421	931		ATT T-	17	
tRNA-Leu	L	12442 12506	65	TAG		4		L	12465 12529	65	TAG	4			L	12447 12511	65	TAG		25	
16S ribosomal RNA	L	12507 13779	1273			0		L	12530 13802	1273		0			L	12547 13782	1236			35	
tRNA-Ile	L	13780 13849	70	TAC		0		L	13804 13873	70	TAC	1			L	13786 13856	71	TAC		3	
12S ribosomal RNA	L	13850 14653	804			0		L	13874 14677	804		0			L	13857 14662	806			0	
control region	L	14654 15796	1143			0		L	14675 15805	1131					L	14660 15774	1115			-3	

* T₁ represents incomplete stop codons. † Intergenic bp indicates gap nucleotides (positive value) or overlapped nucleotides (negative value) between two adjacent genes. ‡ H and L indicate genes transcribed on the heavy and light strands, respectively.

* L, represents incomplete stop codons. † Intergenic bp indicates gap nucleotides (positive value) or overlapped nucleotides (negative value) between two adjacent genes. ‡ H and L indicate genes transcribed on the heavy and light strands, respectively.

Protein-coding genes and codon usage

The length of the total PCGs is 11,133 bp for *A. horsfieldi*, 11,130 bp for *A. chinensis* and 11,092 bp for *A. glabripennis*, accounting for 70.48%, 70.42% and 70.25% of their total mitogenomes, respectively (Table II). Four PCGs (ND1, ND4, ND4L and ND5) are from N strand, and the remaining genes are found on J strand (Fig. 1 and Table I). Thirteen PCGs showed variable range from 156 bp (ATP8) to 1711/1714 bp (ND5) in *A. horsfieldi*, *A. chinensis* and *A. glabripennis* (Table I). All three mitogenomes showed similar characteristics with the smallest size of ATP8 and the largest that of ND5 among PCGs (Fang *et al.*, 2016; Li *et al.*, 2016). In addition, their AT skewness and GC skewness are negative and positive, respectively which are not consistent with those of complete mitogenome (Table II).

Almost all PCGs of three species start with the ATN codon, only ND5 in *A. chinensis* starts with the GTG codon (Table II). In *A. horsfieldi*, an incomplete stop codon “T–” is used for COXI, COXII, COXIII, ND3, ND4 and ND5, while ND1 used TAG as stop codon, and the remaining five PCGs used TAA as stop codon (Table I). In *A. chinensis*, an incomplete stop codon “T” is used for COXI, COXII, ND3, ND4 and ND5, while ND1 and Cytb used TAG as a complete stop codon, and the remaining six PCGs used TAA as complete stop codon (Table I). Moreover, in *A. glabripennis* an incomplete stop codon “T–” is used for COXI, COXII, COXII, ND1, ND3, ND4 and ND5, while TAG is used as a complete stop codon for Cytb, and TAA is used as a complete stop codon for the remaining five PCGs (Table I). In all three mitochondrial genomes, the termination codon TAA appeared higher than TAG and at least five incomplete stop codons “T–” were present (Table I) (Fang *et al.*, 2016; Li *et al.*, 2016). The incomplete termination codons could be added as TAA by post-transcriptional polyadenylation during the mRNA process maturation (Ojala *et al.*, 1981; Schuster and Stern, 2009; Guindon *et al.*, 2010; Ronquist *et al.*, 2012). And this phenomenon has also been discovered in other insects as well (James and Andrew, 2006; Larkin *et al.*, 2007; Wang *et al.*, 2013; Wu *et al.*, 2014; Huang *et al.*, 2015; Liu *et al.*, 2018).

The similar amino acid base composition and the relative synonymous codon usage (RSCU) of the three *Anoplophora* species are found (Fig. 2 and Table III). The genome-wide bias towards AT was viewed to reflect in the codon usage by the PCGs. The total number of codons of the PCGs ranges from 3695 to 3709 (Table II). UUA (Leu2), AUU (Ile), UUU (Phe) and AUA (Met) are the most frequently utilized amino acids (Fig. 3), all high frequency codons are composed of A or U. The third codon frequency of A/T is significantly

higher than that of G/C, reflecting nucleotide A + T bias in the mitochondrial PCGs among Cucujiformia. The composition of most commonly used amino acids, like Leu, Ile, and Phe, varied from 42.47% (*A. glabripennis*) to 43.12% (*A. horsfieldi*) (Fig. 3). This pattern with rich with A or T nucleotides in all PCGs was also similar to other Cucujiformia insects (Kim *et al.*, 2009; Du *et al.*, 2016; Wang and Tang, 2018).

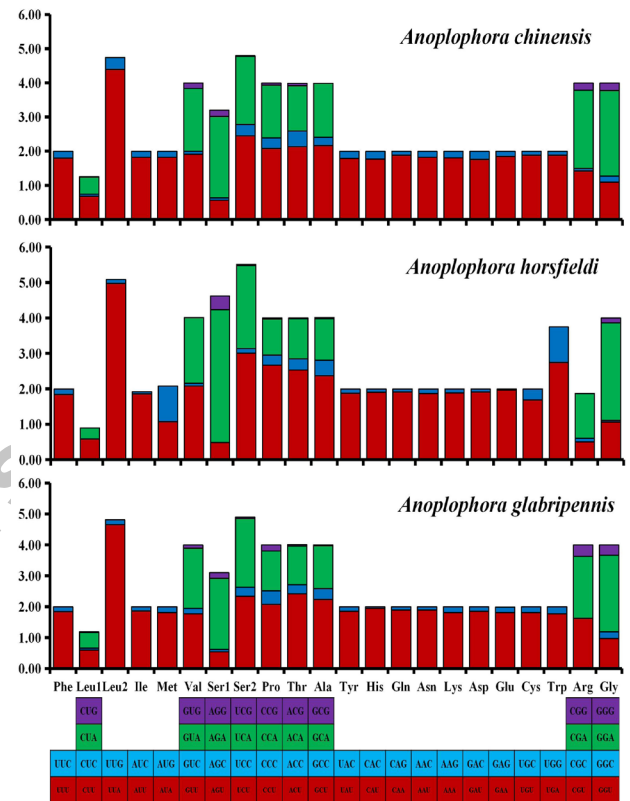


Fig. 2. The relative synonymous codon usage (RSCU) in the mitogenomes of three *Anoplophora* species. Codon families are provided on the x-axis along with the different combinations of synonymous codons that code for that amino acid. RSCU are provided on the y-axis.

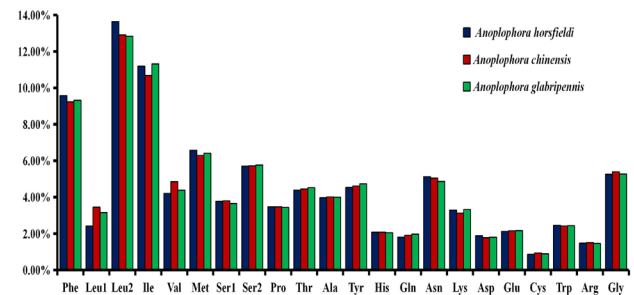


Fig. 3. Total codons in three *Anoplophora* mitogenomes.

Table II. Nucleotide composition and skew values in *Anoplophora horsfieldi*, *Anoplophora chinensis* and *Anoplophora glabripennis*.

	Size (bp)	A (bp)	T (bp)	G(bp)	C (bp)	T %	C %	A %	G %	A+T %	AT skew	GC skew
<i>Anoplophora horsfieldi</i>												
Whole genome	15796	6277	6251	1298	1970	39.57	12.47	39.74	8.22	79.31	0.002	-0.206
13 Protein-coding genes	11133	3730	4987	1220	1196	44.79	10.74	33.50	10.96	78.30	-0.144	0.010
1 st	3711	1274	1372	647	418	36.97	11.26	34.33	17.43	71.30	-0.037	0.215
2 nd	3711	781	1766	513	651	47.59	17.54	21.05	13.82	68.63	-0.387	-0.119
3 th	3711	1675	1849	60	127	49.82	3.42	45.14	1.62	94.96	-0.049	-0.358
Protein-coding genes-H strand	7773	2668	3288	816	1001	42.30	12.88	34.32	10.50	76.62	-0.104	-0.102
1 st	2591	925	864	442	360	33.35	13.89	35.70	17.06	69.05	0.034	0.102
2 nd	2591	611	1157	331	492	44.65	18.99	23.58	12.77	68.24	-0.309	-0.196
3 th	2591	1132	1267	43	149	48.90	5.75	43.69	1.66	92.59	-0.056	-0.552
Protein-coding genes-L strand	8016	2998	3563	690	765	44.45	9.54	37.40	8.61	81.85	-0.086	-0.052
1 st	2672	982	1142	301	247	42.74	9.24	36.75	11.26	79.49	-0.075	0.099
2 nd	2672	829	1210	276	357	45.28	13.36	31.03	10.33	76.31	-0.187	-0.128
3 th	2672	1187	1211	113	161	45.32	6.03	44.42	4.23	89.75	-0.010	-0.175
tRNA genes	1437	586	547	129	175	38.07	12.18	40.78	8.98	78.84	0.034	-0.151
rRNA genes	2076	848	831	122	275	40.03	13.25	40.85	5.88	80.88	0.010	-0.385
Control region	1143	502	486	35	120	42.52	10.50	43.92	3.06	86.44	0.016	-0.548
<i>Anoplophora chinensis</i>												
Whole genome	15805	6240	6032	1388	2145	38.17	13.57	39.48	8.78	77.65	0.017	-0.214
13 Protein-coding genes	11130	3655	4843	1316	1316	43.51	11.82	32.84	11.82	76.35	-0.140	0.000
1 st	3710	1238	1336	675	461	36.01	12.43	33.37	18.19	69.38	-0.038	0.188
2 nd	3710	776	1757	522	655	47.36	17.65	20.92	14.07	68.27	-0.387	-0.113
3 th	3710	1641	1750	119	200	47.17	5.39	44.23	3.21	91.40	-0.032	-0.254
Protein-coding genes-H strand	7785	2654	3157	857	1117	40.55	14.35	34.09	11.01	74.64	-0.087	-0.132
1 st	2595	900	834	463	398	32.14	15.34	34.68	17.84	66.82	0.038	0.075
2 nd	2595	611	1163	331	490	44.82	18.88	23.55	12.76	68.36	-0.311	-0.194
3 th	2595	1143	1160	63	229	44.70	8.82	44.05	2.43	88.75	-0.007	-0.568
Protein-coding genes-L strand	7998	2863	3571	1031	533	44.65	6.66	35.80	12.89	80.45	-0.110	0.318
1 st	2666	966	1115	413	172	44.65	6.66	35.80	12.89	80.45	-0.110	0.318
2 nd	2666	785	1213	386	282	45.50	10.58	29.44	14.48	74.94	-0.214	0.156
3 th	2666	1112	1243	232	79	46.62	2.96	41.71	8.70	88.33	-0.056	0.492
tRNA genes	1446	579	539	181	147	37.28	10.17	40.04	12.52	77.32	0.036	0.104
rRNA genes	2076	810	851	287	128	40.99	6.17	39.02	13.82	80.01	-0.025	0.383
Control region	1131	473	495	104	59	43.77	5.22	41.82	9.20	85.59	-0.023	0.276
<i>Anoplophora glabripennis</i>												
Whole genome	15768	6248	6104	1355	2061	38.71	13.07	39.62	8.59	78.34	0.012	-0.207
13 Protein-coding genes	11092	3676	4867	1277	1272	43.88	11.47	33.14	11.51	77.02	-0.139	0.002
1 st	3698	1262	1336	653	447	36.13	12.09	34.13	17.66	70.25	-0.028	0.187
2 nd	3698	781	1752	509	656	47.38	17.74	21.12	13.76	68.50	-0.383	-0.126
3 th	3696	1633	1779	115	169	48.13	4.57	44.18	3.11	92.32	-0.043	-0.190
Protein-coding genes-H strand	8863	3153	3678	876	1156	41.50	13.04	35.57	9.88	77.07	-0.077	-0.138
1 st	2954	1076	1004	451	423	33.99	14.32	36.43	15.27	70.41	0.035	0.032
2 nd	2955	776	1328	341	510	44.94	17.26	26.26	11.54	71.20	-0.262	-0.199
3 th	2954	1301	1346	84	223	45.57	7.55	44.04	2.84	89.61	-0.017	-0.453
Protein-coding genes-L strand	6812	2391	3044	901	476	44.69	6.99	35.10	13.23	79.79	-0.120	0.309
1 st	2271	793	967	364	147	42.58	6.47	34.92	16.03	77.50	-0.099	0.425
2 nd	2271	635	1049	325	262	46.19	11.54	27.96	14.31	74.15	-0.246	0.107
3 th	2270	963	1028	212	67	45.29	2.95	42.42	9.34	87.71	-0.033	0.520
tRNA genes	1431	572	545	173	141	38.09	9.85	39.97	12.09	78.06	0.024	0.102
rRNA genes	2039	794	833	285	127	40.85	6.23	38.94	13.98	79.79	-0.024	0.383
Control region	1115	503	478	42	92	42.87	8.25	45.11	3.77	87.98	0.025	-0.373

Table III. The codon number and relative synonymous codon usage in mitochondrial protein coding genes.

Ami- no acid	Codon	Count			RSCU			Frequency (%)			Ami- no acid	Codon	Count			RSCU			Frequency (%)		
		AH	AC	AG	AH	AC	AG	AH	AC	AG			AH	AC	AG	AH	AC	AG	AH	AC	AG
Phe	UUU(F)	326	308	316	1.84	1.80	1.84	8.79%	8.32%	8.56%	Tyr	UAU(Y)	158	153	162	1.88	1.79	1.85	4.27%	4.13%	4.39%
Phe	UUC(F)	29	34	28	0.16	0.20	0.16	0.78%	0.92%	0.76%	Tyr	UAC(Y)	10	18	13	0.12	0.21	0.15	0.27%	0.49%	0.35%
Leu	UUA(L)	495	444	459	4.98	4.40	4.66	13.35%	11.99%	12.44%	Stop	UAA(*)	6	6	5	0.19	1.50	1.67	0.16%	0.16%	0.14%
Leu	UUG(L)	11	34	15	0.11	0.34	0.15	0.30%	0.92%	0.41%	Stop	UAG(*)	2	2	1	0.06	0.50	0.33	0.05%	0.05%	0.03%
Leu	CUU(L)	58	69	59	0.58	0.68	0.60	1.56%	1.86%	1.60%	His	CAU(H)	73	68	74	1.90	1.77	1.95	1.97%	1.84%	2.00%
Leu	CUC(L)	1	7	6	0.01	0.07	0.06	0.03%	0.19%	0.16%	His	CAC(H)	4	9	2	0.10	0.23	0.05	0.11%	0.24%	0.05%
Leu	CUA(L)	31	51	50	0.31	0.50	0.51	0.84%	1.38%	1.35%	Gln	CAA(Q)	64	67	69	1.91	1.89	1.89	1.73%	1.81%	1.87%
Leu	CUG(L)	0	1	2	0.00	0.01	0.02	0.00%	0.03%	0.05%	Gln	CAG(Q)	3	4	4	0.09	0.11	0.11	0.08%	0.11%	0.11%
Ile	AUU(I)	403	361	388	1.86	1.82	1.86	10.87%	9.75%	10.51%	Asn	AAU(N)	178	170	170	1.87	1.82	1.89	4.81%	4.59%	4.61%
Ile	AUC(I)	12	35	30	0.06	0.18	0.14	0.32%	0.95%	0.81%	Asn	AAC(N)	12	17	10	0.13	0.18	0.11	0.32%	0.46%	0.27%
Ile	AUA(M)	234	212	216	1.08	1.82	1.82	6.31%	5.73%	5.85%	Lys	AAA(K)	115	105	112	1.89	1.81	1.82	3.11%	2.84%	3.03%
Met	AUG(M)	10	21	21	1.00	0.18	0.18	0.27%	0.57%	0.57%	Lys	AAG(K)	7	11	11	0.11	0.19	0.18	0.19%	0.30%	0.30%
Val	GUU(V)	81	86	72	2.08	1.91	1.78	2.19%	2.32%	1.95%	Asp	GAU(D)	67	58	62	1.91	1.76	1.85	1.81%	1.57%	1.68%
Val	GUC(V)	3	4	7	0.08	0.09	0.17	0.08%	0.11%	0.19%	Asp	GAC(D)	3	8	5	0.09	0.24	0.15	0.08%	0.22%	0.14%
Val	GUA(V)	72	83	79	1.85	1.84	1.95	1.94%	2.24%	2.14%	Glu	GAA(E)	78	74	73	1.97	1.85	1.82	2.11%	2.00%	1.98%
Val	GUG(V)	0	7	4	0.00	0.16	0.10	0.00%	0.19%	0.11%	Glu	GAG(E)	1	6	7	0.03	0.15	0.17	0.03%	0.16%	0.19%
Ser	UCU(S)	116	108	102	3.01	2.45	2.34	3.13%	2.92%	2.76%	Cys	UGU(C)	27	33	30	1.69	1.89	1.82	0.73%	0.89%	0.81%
Ser	UCC(S)	5	15	13	0.13	0.34	0.30	0.13%	0.41%	0.35%	Cys	UGC(C)	5	2	3	0.31	0.11	0.18	0.14%	0.05%	0.08%
Ser	UCA(S)	90	88	96	2.34	1.99	2.21	2.43%	2.38%	2.60%	Stop	UGA(W)	87	85	80	2.75	1.89	1.78	2.35%	2.30%	2.17%
Ser	UCG(S)	1	1	2	0.03	0.02	0.05	0.03%	0.03%	0.05%	Trp	UGG(W)	4	5	10	1.00	0.11	0.22	0.11%	0.14%	0.27%
Pro	CCU(P)	86	67	66	2.67	2.08	2.08	2.32%	1.81%	1.79%	Arg	CGU(R)	15	20	22	0.51	1.43	1.63	0.41%	0.54%	0.60%
Pro	CCC(P)	9	10	14	0.28	0.31	0.44	0.24%	0.27%	0.38%	Arg	CGC(R)	3	1	0	0.10	0.07	0.00	0.08%	0.03%	0.00%
Pro	CCA(P)	33	50	41	1.02	1.55	1.29	0.89%	1.35%	1.11%	Arg	CGA(R)	37	32	27	1.26	2.29	2.00	1.00%	0.86%	0.73%
Pro	CCG(P)	1	2	6	0.03	0.06	0.19	0.03%	0.05%	0.16%	Arg	CGG(R)	0	3	5	0.00	0.21	0.37	0.00%	0.08%	0.14%
Thr	ACU(T)	103	88	101	2.53	2.13	2.42	2.78%	2.38%	2.74%	Ser	AGU(S)	19	25	24	0.49	0.57	0.55	0.51%	0.68%	0.65%
Thr	ACC(T)	13	19	12	0.32	0.46	0.29	0.35%	0.51%	0.33%	Ser	AGC(S)	0	3	3	0.00	0.07	0.07	0.00%	0.08%	0.08%
Thr	ACA(T)	46	55	52	1.13	1.33	1.25	1.24%	1.49%	1.41%	Arg	AGA(S)	110	105	100	3.75	2.38	2.30	2.97%	2.84%	2.71%
Thr	ACG(T)	1	3	2	0.02	0.07	0.05	0.03%	0.08%	0.05%	Arg	AGG(S)	11	8	8	0.38	0.18	0.18	0.30%	0.22%	0.22%
Ala	GCU(A)	87	81	83	2.37	2.17	2.24	2.35%	2.19%	2.25%	Gly	GGU(G)	52	55	48	1.07	1.10	0.98	1.40%	1.49%	1.30%
Ala	GCC(A)	16	9	13	0.44	0.24	0.35	0.43%	0.24%	0.35%	Gly	GGC(G)	2	9	10	0.04	0.18	0.21	0.05%	0.24%	0.27%
Ala	GCA(A)	43	59	51	1.17	1.58	1.38	1.16%	1.59%	1.38%	Gly	GGA(G)	134	125	121	2.75	2.50	2.48	3.62%	3.38%	3.28%
Ala	GCG(A)	1	0	1	0.03	0.00	0.03	0.03%	0.00%	0.03%	Gly	GGG(G)	7	11	16	0.14	0.22	0.33	0.19%	0.30%	0.43%

RSCU, relative synonymous codon usage; AH, *Anoplophora horsfieldi*; AC, *Anoplophora chinensis* (NC_029230); AG, *Anoplophora glabripennis* (NC_008221).

Ribosomal and transfer RNA genes

Twenty-two tRNAs of *A. horsfieldi*, *A. chinensis* and *A. glabripennis* mitogenomes scattered discontinuously across the complete mitogenome (Table I). The tRNAs length of these three species were 1,437, 1,446 and 1,431 bp, respectively, and accounted for 9.10%, 9.15% and 9.08% of the total mitogenomes, respectively (Table I). The three mitogenomes possess 22 tRNA genes, eight

transcribed from N strand and 14 from J strand (Fig. 1, Table I). The length of these 22 tRNAs range from 60 (trnR) to 76 bp (trnD) in *A. horsfieldi*, from 62 (trnC) to 84 bp (trnD) in *A. chinensis* and from 62 (trnH) to 74 bp (trnP) in *A. glabripennis* (Table I). 21 of 22 tRNAs can form typical cloverleaf secondary structure, while trnS1 (AGN) formed a simple loop due to lacking the DHU arm (Fig. 4, Supplementary Figs. S1-S2), as discovered in other

coleopteran species (Stewart and Beckenbach, 2003; Song *et al.*, 2010; Cabrera-Brandt and Gaitan-Espitia, 2015). The lack of DHU stem in trnS1 is a common phenomenon in insect mitogenomes (Beckenbach, 2011; Cameron, 2014; Li *et al.*, 2015), and has been proven as a typical characteristic of metazoan mitogenomes (Wang *et al.*, 2019). Many nucleotide substitutions are spotted in four different stems and the anticodon loop is highly conserved (Fig. 4). Besides the orthodox AU and CG pairs, some mismatched base pairs are also discovered in different stems. A total of 15 GU mismatches, 3 UU mismatches, and 1 AG mismatch are found in *A. horsfieldi* (Fig. 4), 17 GU mismatches, 2 UU mismatches, 2 AG mismatches, and 1 AA mismatch are found in *A. glabripennis* while 17 GU mismatches, 2 UU mismatches, 1 AG mismatch, and 1 AC mismatches are found in *A. chinensis* (Fig. 4, Supplementary Figs. S1-S2, Table I).

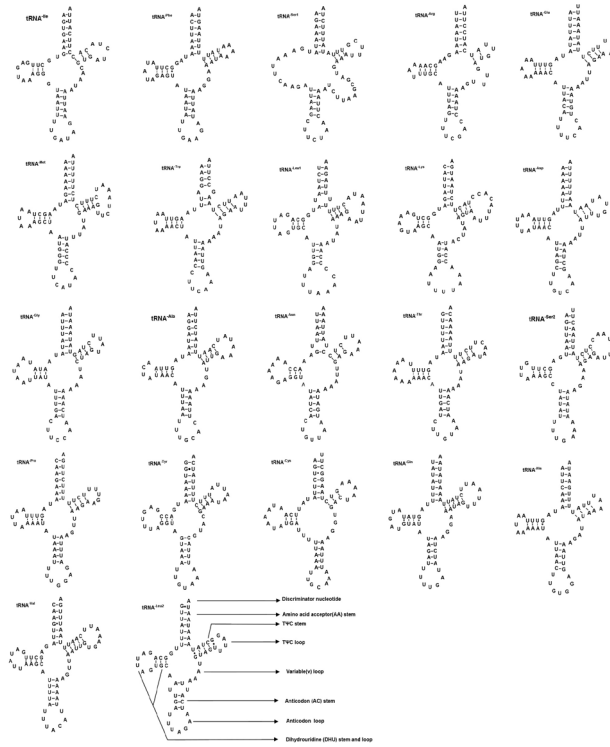


Fig. 4. Putative tRNA secondary structures predicted from the 22 tRNA gene sequences found in the *Anoplophora horsfieldi* mitogenome.

Two rRNA genes (rrnL and rrnS) are encoded by the N-strand in *A. horsfieldi*, *A. chinensis* and *A. glabripennis* and rrnL is distributed between trnL1 and trnV, while rrnS lies between trnV and CR. The genes rrnL and rrnS are 1273 and 804 bp long in *A. horsfieldi* and *A. chinensis*, and 1236 and 806 bp long in *A. glabripennis*, respectively

(Table I). The lengths range from 1236 to 1273 bp in rrnL, and from 804 to 806 bp in rrnS in these three mitogenomes of genus *Anoplophora* (Fang *et al.*, 2016; Li *et al.*, 2016). The A+T content of the two rRNAs (80.88%) in *A. horsfieldi* was higher than 80.01% in *A. chinensis* and 79.79% in *A. glabripennis*, with positive AT skew (0.010) and negative GC skew (-0.385), however, in the other two species, the AT and GC skews are negative and positive, respectively (Table III).

Overlapping and intergenic spacer regions

A total of 15 gene overlap in the *A. horsfieldi* mitogenome from 1 to 13 bp, adding up to 59 bp, 14 gene overlaps exist in the *A. chinensis* mitogenome with the length from 1 to 8 bp, amounting to 45 bp, while 18 gene overlaps occur in the *A. glabripennis* mitogenome with the length from 1 to 9 bp, adding up to 64 bp (Table I). The longest overlap region (13 bp) of the three mitogenomes is found between trnK and trnD. All three *Anoplophora* species have eight identical overlap regions, including trnQ-trnM (1 bp), ATP6-COXIII (1 bp), trnE-trnF (1 bp), ND6-Cytb (1 bp), Cytb-trnS (2 bp), ND4-ND4L (4 bp), ATP8-ATP6 (7 bp), trnY-COXI (8 bp) and trnW-trnC (8 bp) (Coates, 2014; Fang *et al.*, 2016; Li *et al.*, 2016; Yao *et al.*, 2017). Eight intergenic spacers appear in *A. horsfieldi*, ranging in length from 2 to 17 bp and amounting to 42 bp. Nine intergenic spacers occur in *A. chinensis*, ranging in size from 1 to 17 bp and adding up to 41 bp. A total of 115 bp in *A. glabripennis* distributed in 12 intergenic spacers, ranging in size from 1 to 35 bp. All three mitochondrial genomes share four identical intergenic spacers (trnR-trnN (2 bp), ND4L-trnT (2 bp), ND5-trnS (3 bp), trnS-ND1 (17 bp)) (Table I). The size of the intergenic spacers is more variable than overlaps.

Two 7-bp long overlaps (ATGATAA) were discovered in the *Anoplophora* species, which were also observed in other Polyphaga insects (Fig. 5), however, 7-bp long overlaps (ATGATTA) was discovered in *Epicauta chinensis* (KP692789) and 7-bp long overlaps (ATGATAG) was found in *Tribolium castaneum* (AJ312413) (Friedrich and Muqim, 2003) and *Cryptolestes pusillus* (NC_028204) (Li *et al.*, 2016) between ATP8 and ATP6. The overlaps lie between ATP8 and ATP6 on the J-strand and between ND4L and ND4 on the N-strand, respectively. The overlapped sequences were translated as a bicstron (Stewart and Beckenbach, 2005; Wang and Tang, 2018). The other two 5 bp (TTAAT) and 7 bp (TTTAGT) long motif were detected between trnSer (UCN) and ND1 in mitogenomes of the three *Anoplophora* species, which was also present in other cucujiform beetle (Fig. 6). However, another 5 bp long motif (TAGTA) was found at this location (Wang and Tang, 2018). This has been explained as the possible

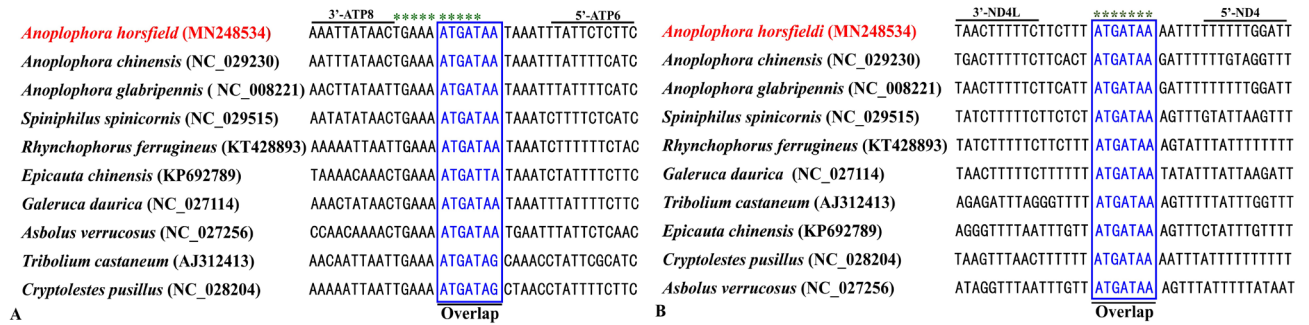


Fig. 5. Alignment of overlapping region across Cucujiformia and other insects. (A) Atp8 and Atp6, the motifs “ATGATA/TA/G” in the box are conservative; (B) ND4L and ND4, the motifs “ATGATAA” in the box are conservative.

binding site of mtTERM because it is located at the end of the H-strand region in the circular mitochondrial genome (Taanman, 1999). Based on mitogenomic comparisons, the motifs between ATP8 and ATP6, and between trnSer and ND1 were relatively conserved in four suborders (Polyphaga, Adephaga, Archostemata and Myxophaga). However, the motif “ATGATAA” was only found between ND4 and ND4L of the mitogenomes of Polyphaga insects, while “ATGTAA” was found in the other three suborders’ mitogenomes (Adephaga, Archostemata and Myxophaga) (Wang and Tang, 2018).

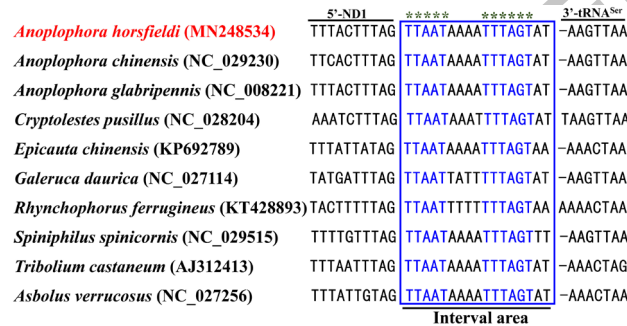


Fig. 6. Alignment of the intergenic spacer region between trnS (UCN) and ND1 of several Cucujiformia insects. The shaded “TTAAT” and “TTTAGT” motif is conserved across the Cucujiformia species.

A+T-rich region

The A+T-rich region in insect mitochondrial genome, similar to the control region of vertebrate mitochondrial genome, possess the transcription and replication origin sites (Andrews *et al.*, 1999; Yukuhiro *et al.*, 2002; Zhu *et al.*, 2013). The largest A+T-rich region lied between the genes rrnS and trnI. This region plays important roles in regulating transcription and replication (Zhang and Hewitt, 1997). The lengths of A+T-rich region are 1,143 bp in *A. horsfieldi*. In all three *Anoplophora* species, *A. glabripennis* has the smallest

control region of 1,115 bp, while *A. horsfieldi* has the largest that of 1,143 bp (Fig. 7, Table I). The A + T contents are 86.44% in *A. horsfieldi*, 85.59% in *A. chinensis* and 87.98% in *A. glabripennis*. The motif of tandem repeats in A+T-rich region has been recorded in many insect orders (Zhang and Hewitt, 1997; Du *et al.*, 2017; Wang *et al.*, 2018; Huang *et al.*, 2019; Li *et al.*, 2019), but is found in *A. horsfieldi* for the first time. *A. horsfieldi* and *A. glabripennis* have three types of long repeat tandem units and one types of microsatellite-like repeat, while there are only two different long repeats in *A. chinensis* (Fig. 7B). The repeat units presented obvious differences in size and copy number among Coleoptera species, thus resulting in various lengths of the A+T-rich region.

Tandem repeats (due to difference in type and number) can be used as microsatellites for phylogeography and population genetics researches and also as a molecular marker in evolutionary biology (Wang *et al.*, 2018; Zhang *et al.*, 2019). In addition, in some insect species (Kim *et al.*, 2006; Yin *et al.*, 2012), there was no long tandem repetitive sequence in this region, but it did have some microsatellite-like repeats (such as, (A)₉, (T)₁₅, (C)₈, (TA)₆, (TA)₈). The A+T-rich region in *A. horsfieldi* possess a 16 bp long poly-T and 14 bp long poly-A stretches which has been considered as a possible recognition site for the initiation of replication of the mtDNA minor strand (Fig. 7A, Andrews *et al.*, 1999; Kim *et al.*, 2009; Du *et al.*, 2016). When compared with the A+T-rich region of three *Anoplophora* species, three conserved sequence blocks (CSBs) were recognized in those species. These CSBs ranged in length from 23 to 111 bp, and their sequence similarity among species was generally more than 50%. These CSBs were also found in other stoneflies mitogenomes (Qian *et al.*, 2014; Chen and Du, 2015, 2017; Cao *et al.*, 2019), but the functions and characteristics of these conserved blocks need to be further studied. In *A. horsfieldi* and *A. glabripennis*, the AT and GC skewness of the A+T-rich region are negative and positive, respectively, while they are opposite in *A. chinensis* (Table II).

rrnS-14,654-AAAAAAAAAAGAACTACTCCCCCTATTAAAAATATTCAAACCTCATTTTTTTAGCACTAA
 AACTTTCTATATAAAATTATTTCAATATCCCCTAAATACTGATTTTCATAAACTTCCATAAAAC
 TCATTTATAAAATTATTTCAATATCCCCTAAATACTGATTTTCATAAACTTCCATAAAACTCAT
 TTATAAAATTATTTCAATATCCCCTAAATACTGATTTTCATAAACTTCCATAAAACTCATTAT
 AAAATTATTTCAATATCCCCTAAATACTGATTTTCATAAACTTCCATAAAACTCATTATATAAA
 TTATTTCAATATCCCCTAAATACTGATTTTCATAAACTTCCATAAAACTCATTATATAAAATTAT
 TTCAATATCCCCTAAATACTGATTTTCATAAACTTCCATTTAAATATAATAATAATTTTATAAA
CATATTATTATATAAAATTTAATATTTTATATAATTAAAAATAATTATTTAATAAAATTATAATTTT
 ATATTATATCATTTTTTTATACTAATATAAAAAAAGGGTAGTTTTTTTTTTTTTTTTAAAAATTGTT
 ATTTTCAATAACAAATTTATATATTAATAATATATAAATTATTTATATATATAACAATTATATT
 ATATATATTACTAAATATATTATATATATAAGTATATATTATTTTTATATTTATATTAAATTGTT
ATATATATATAAGATATTTATATATCTATAATAATATAATATAAATGTATATATATAAATAATAAA
ATAAATAATATTGATTCATTATAGAAATAATTAATACTAATATAAATTTCTATTATCAATTTTATTT
 TTTTCATTATTGATTTATTTAAAAATAGTATTATTCATAAAATTTTATTAATATCTGATAAAAAATA
 GGAGATCCTTATATTAATATAAAATAGCTTATATAAAATATAAACTGATTAGGCATATATTAATAT
 AATTTTTATTTTAAACAAAAAATAAAAAAAAAAAAAAATGTAAAAAAATACAATCATTGCATTTTCT
 TTTAGTTAATTTTTTTTTCGCTAAAACCCAATTTTACAAAAATTTACATTAAATTCATGTTTATTTA
 ATTTAATAACTAAAAAAGTTAT-15,796-*trnI*

A

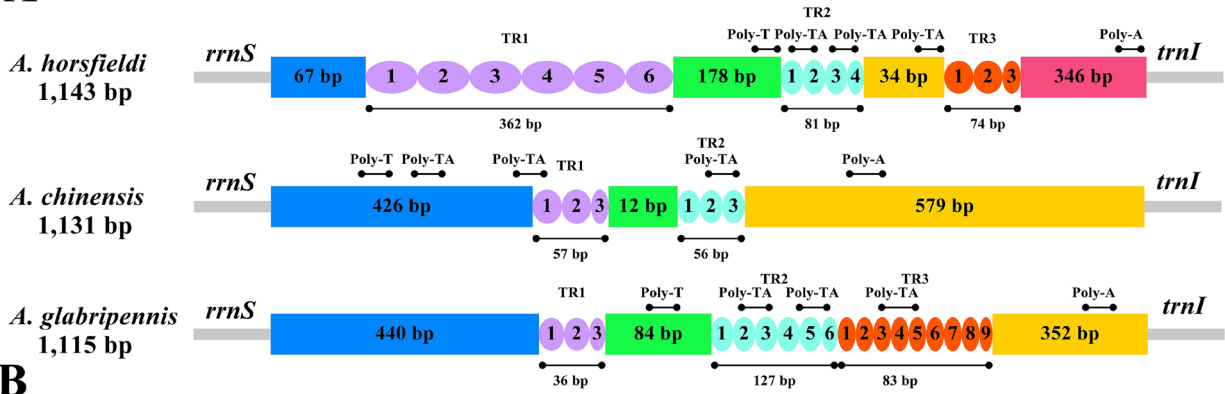


Fig. 7. (A) Organization of the A+T-rich regions in the *Anoplophora horsfieldi* mitogenomes. The square box represents the feature sequence CSB1-3. The poly-T stretch is underlined while the poly-A stretch is double underlined. The single microsatellite T/A repeats sequence are indicated by single underlining. (B) The colored ovals indicate the tandem repeats; the remaining regions are shown with different color boxes. TR1-3 represents different types of repeat sequences. Horizontal lines with solid dots at both ends represent the structures of poly (A), poly (T) and poly (TA), respectively.

Phylogenetic analyses

The phylogenetic analyses were carried out using the concatenated nucleotide sequences of 13 PCGs from 117 Cucujiformia mitogenomes (Supplementary Table S1). Similar topologies were achieved by using Bayesian (BI) and maximum likelihood (ML) analyses (Fig. 8). Phylogenetic analysis showed that *A. horsfieldi* grouped with *A. glabripennis* and *A. chinensis* with high nodal

supports. In *Anoplophora*, *A. horsfieldi* is at the base of the genus. In our phylogenetic analyses, *Anoplophora* was clustered with *Monochamus*, rather than genus *Psacotha* and it was consistent with the results of previous phylogenetic analyses based on mitochondrial data (Coates, 2014; Yuan *et al.*, 2016; Liu *et al.*, 2018; Wang *et al.*, 2019). In addition to *Orsodacne lineola* (Vesperidae), Prioninae, Cerambycinae, Disteniinae, Cassidinae and

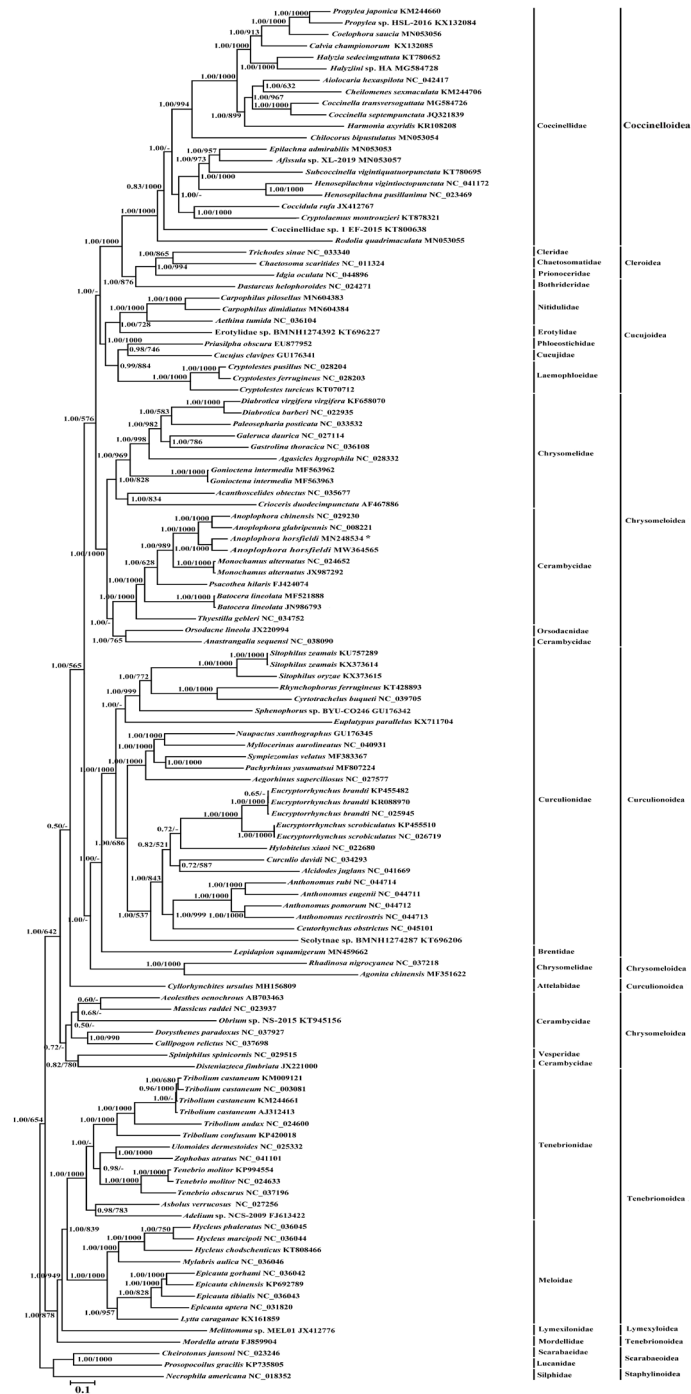


Fig. 8. Phylogenetic tree of the relationships among 117 mitogenome sequences of Cucujiformia based on the nucleotide dataset of the 13 mitochondrial protein-coding genes of 11,154 nucleotides with *Prosopocoilus gracilis* (Coleoptera: Scarabaeiformia: Lucanidae: KP735805), *Cheirotonus jansoni* (Coleoptera: Scarabaeiformia: Scarabaeidae: NC_023246) and *Necrophila americana* (Coleoptera: Staphyliniformia: Silphidae: NC_018352) as outgroups. Branch lengths and topology are from the BI analysis. Numbers above branches specify posterior probabilities from Bayesian inference (BI) and bootstrap percentages from maximum likelihood (ML, 1000 replications) analyses. The GenBank numbers and scientific names of all species are shown in [Supplementary Table S1](#). Tree topologies produced by Bayesian inferences (BI) and maximum likelihood (ML) analyses were equivalent. Bayesian posterior probability and bootstrap support values for ML analyses are shown orderly on the nodes. The asterisks indicate new sequences generated in this study.

Philinae species, Cerambycidae is closely related with family Chrysomelidae, and *Anastrangalia sequensi* was positioned as the basal lineage of Cerambycidae (Fig. 8). At the same time, the phylogenetic topological structure supports the two families as monophyletic. However, comparing our results to previous morphological and molecular analyses of Cerambycidae and Chrysomelidae relationships was harder to determine (Marvaldi et al., 2009; Yuan et al., 2016; Liu et al., 2018). Previous phylogenetic analyses of molecular data resulted in a monophyletic Cerambycidae and Chrysomelidae (Zhou et al., 2016; Liu et al., 2017; Yao et al., 2017). This may be because of the limited mitogenomic data, especially for Chrysomelidae species. Based on phylogenetic tree, the monophyly of Chrysomelidae and Cerambycidae cannot be supported in Chrysomeloidea, and their evolutionary relationships are relatively complex (Fig. 8) and need to be confirmed by additional samples and species and phylogenetic information, including nuclear genes.

Previously, the series Cucujiformia insects contain six superfamilies (Bouchard et al., 2011; Lawrence et al., 2011). However, our results support that Cucujiformia is divided into seven superfamilies, which is in agreement with the views of Robertson et al. (2015) and Yuan et al. (2016). Although the topological structures of phylogenetic trees are different among different data strings and analysis methods, these studies do not support the previous classification of Cucujoidea as monophyletic (Hunt et al., 2007; Marvaldi et al., 2009; Lawrence et al., 2011; Bocak et al., 2014; Robertson et al., 2015; Timmermans et al., 2016), which is consistent with our phylogenetic results (Fig. 8), and found that Elateroidea is embedded between Coccinelloidea and Cleroidea classification. Recently, Robertson et al. (2015) constructed a high-level phylogenetic analysis for Cucujoidea according to four mitochondrial and four nuclear genes, where two superfamilies (Cleroidea and Cucujoidea) were redefined and a new superfamily Coccinelloidea (cerylonid series) was proposed, and our reconstructed phylogenetic results also support the Coccinelloidea as a new superfamily (Fig. 8). In these seven superfamilies, except for *Dastarcus helophoroides* and *Cyllorhynchites ursulus*, five superfamilies (Lymexyloidea, Cucujoidea, Coccinelloidea, Cleroidea and Curculionoidea) are consistently supported as monophyletic with ML and BI methods, which is in accord with the results of Timmermans et al. (2016) and Yuan et al. (2016) (Fig. 8). Although the monophyly of other two superfamilies (Chrysomeloidea and Coccinelloidea) are recovered in PhyloBayes analyses with the P123R dataset, our results support that Chrysomeloidea and Tenebrionoidea belong to paraphyletic relationships based on 13 PCGs (Fig. 8). Yuan et al. (2016) carried

out the PhyloBayes analyses with the P123R dataset, and formed such a cucujiform relationship of (Cleroidea + (Coccinelloidea + ((Lymexyloidea + Tenebrionoidea) + (Cucujoidea + (Chrysomeloidea + Curculionoidea)))) (Fig. 8), however, our topology tree supports such phylogenetic relationships of ((Lymexyloidea + Tenebrionoidea) + (Curculionoidea + (Chrysomeloidea + (Cucujoidea + (Cleroidea + Coccinelloidea)))) (Fig. 8). This is different from the studies of (((Chrysomeloidea + Cucujoidea) + Tenebrionoidea) + Cleroidea) obtained by Kim et al. (2009), (((Curculionoidea + Chrysomeloidea) + Cucujoidea) + (Cucujoidea + Cleroidea)) Tenebrionoidea in the PCG-BI tree obtained by Li et al. (2016), (((Curculionoidea + Chrysomeloidea) + Cucujoidea) + (Cleroidea + (cerylonid lineages + (Lymexyloidea + Tenebrionoidea))) constructed by Timmermans et al. (2016) and (Cleroidea + (Erotylid series + (Tenebrionoidea + (Cucujoidea + (Chrysomeloidea + Curculionoidea)))) of Crampton-Platt et al. (2015) where these superfamily relationships within Cucujiformia cannot be well resolved. Lymexyloidea + Tenebrionoidea were positioned as the basal lineage of the series Cucujiformia insects in the PCG-BI/ML tree (Fig. 8), which was inconsistent with previous reports (Coates, 2014; Li et al., 2016), whereas superfamily Tenebrionoidea is located at the basal position in the ML and BI trees, and is also different from the basal position of Cleroidea in Cucujiformia (Kim et al., 2009; Marvaldi et al., 2009). These differences may be due to the use of different numbers of samples, molecular markers and analytical methods.

From the view of topology tree, the layout of Chrysomeloidea species is complicated and paraphyletic, which is consistent with Bocak et al. (2014). Relationships of some families in Chrysomeloidea remain to be poorly supported, especially for the small families Orsodacnidae and Vesperidae. Besides Prioninae, Cerambycinae, Disteniinae, Cassidinae and Philinae, the phylogenetic relationship of the family Cerambycidae is monophyletic. Unsatisfactory results were also found for internal relationships of Cerambycidae for which the sampling number and density of species were low and especially the representativeness of conservative loci was poor (Bocak et al., 2014). The Prioninae, Cerambycinae, Disteniinae, Cassidinae and Philinae species are not clustered in the family Chrysomelidae, which leads to the formation of paraphyly of the family. Besides the five subfamilies, Chrysomelidae were well supported and this is in accord with previous study results from Gomez-Zurita et al. (2007) and Bocak et al. (2014). The major clades were resolved (Fig. 8), containing the basal splits of a group of Donaciinae, Criocerinae and Bruchinae, the monophyly of Cassidinae. However, our results support the paraphyly of

Galerucinae, Chrysomelinae and Cerambycinae (Fig. 8). The phylogenetic relationships at subfamily Prioninae, Cerambycinae, Disteniinae, Cassidinae and Philinae, especially in family Vesperidae and Orsodacnidae, still have not been resolved due to the lack of sufficient phylogenetic information and many more new species.

In word, as far as Cucujiformia, Chrysomeloidea and Chrysomelidae is concerned, the conflict results about phylogeny might be due to the different types of molecular markers and analytical methods (Kim *et al.*, 2009; Marvaldi *et al.*, 2009; Coates, 2014; Crampton-Platt *et al.*, 2015; Meiklejohn *et al.*, 2014). Therefore, the phylogenetic relationship and taxonomic status within the Cucujiformia insects require a large number of additional samples and more molecular markers to elucidate their evolutionary relationship. Undoubtedly, this study will contribute to explore the phylogeny, systematics and taxonomy of the Cucujiformia insects.

CONCLUSION

In this study, the entire mitogenome of *A. horsfieldi* (16,759 bp) was sequenced and annotated. The mitochondrial gene order and orientation of *A. horsfieldi* were similar to Chrysomeloidea species. The motifs, 'ATGATAA' between ND4L and ND4, was more conserved than that between trnS (UCN) and ND1 and between ATP8 and ATP6 in Cucujiformia. Phylogenetic tree showed that *A. horsfieldi* grouped with *A. glabripennis* and *A. chinensis* with high nodal supports. Within Cucujiformia, such phylogenetic relationships of (Lymexyloidea + (Tenebrionoidea + (Curculionoidea + (Chrysomeloidea + (Cucujoidea + (Cleroidea + Coccinelloidea)))))) were rebuilt, and the paraphyletic relationships that Chrysomeloidea, Coccinelloidea and Curculionoidea were supported. We believe that the mitogenomes of *A. horsfieldi* will help people to further the studies of molecular evolution, and Cucujiformia mitogenome architecture and phylogenetics.

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IRB approval

The animal handling procedures conformed with the China Animal Welfare guidelines and have been approved by the Ethic and Animal Welfare Committee and the Animal Protection and Use Commission of Mianyang Normal University (MYSY2017JC02).

Ethical statement

This study was carried out in accordance with the Animal Care and Use Committee at the Mianyang Normal University. Efforts were taken to minimize suffering and included administering anesthesia. The study did not involve endangered or protected species.

Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20220824100800>

Statement of conflict of interest

The authors have declared no conflict of interest.

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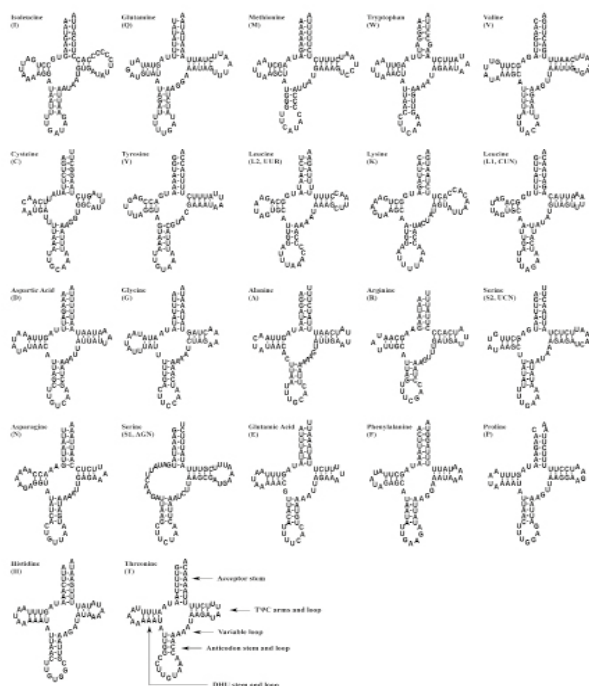
Supplementary Material

Comparative Mitgenome Analysis of *Anoplophora horsfieldi* and Other Chrysomeloidea, Cucujiformia Insects Reveals Conserved Mitogenome Organization and Phylogeny

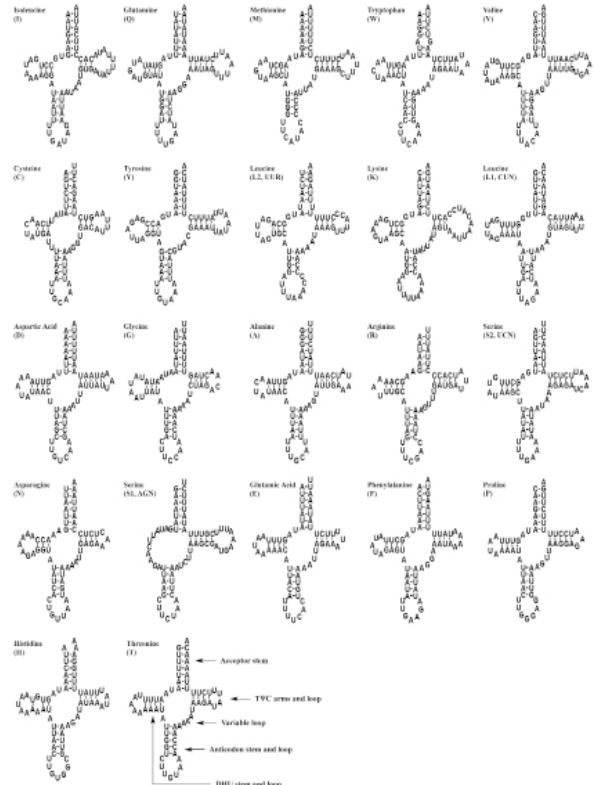
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Supplementary Fig. S1. Putative tRNA secondary structures predicted from the 22 tRNA gene sequences found in the *Anoplophora chinensis* mitogenome.



Supplementary Fig. S2. Putative tRNA secondary structures predicted from the 22 tRNA gene sequences found in the *Anoplophora glabripennis* mitogenome.

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Supplementary Table S1. Species mentioned in the present study with their GenBank accession number.

No. Order	Suborder	Superfamily	Family	Subfamily	Tribes	Genus	Species	Species Accession no.
1 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Lamiini	<i>Anoplophora</i>	<i>Anoplophora horsfieldi</i>	15,796 MN248534
2 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Lamiini	<i>Anoplophora</i>	<i>Anoplophora horsfieldi</i>	15,837 MW364555
3 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Lamiini	<i>Anoplophora</i>	<i>Anoplophora glabripennis</i>	15,774 NC_008221
4 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Lamiini	<i>Anoplophora</i>	<i>Anoplophora chinensis</i>	15,805 NC_029230
5 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Monochamini	<i>Monochamus</i>	<i>Monochamus alternatus</i>	15,874 NC_024652
6 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Monochamini	<i>Monochamus</i>	<i>Monochamus alternatus</i>	14,649 JX987292
7 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Batocerini	<i>Batocera</i>	<i>Batocera lineolata</i>	15,418 JN986793
8 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Batocerini	<i>Batocera</i>	<i>Batocera lineolata</i>	15,420 MF521888
9 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Agrini	<i>Psacotha</i>	<i>Psacotha hilaris</i>	15,856 FJ424074
10 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Saperdini	<i>Thyestilla</i>	<i>Thyestilla gebleri</i>	15,505 NC_034752
11 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Cerambycinae	Cerambycini	<i>Aeolesthes</i>	<i>Aeolesthes oenochrous</i>	15,747 AB703463
12 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Cerambycinae	Cerambycini	<i>Massicus</i>	<i>Massicus raddai</i>	15,858 NC_023937
13 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Cerambycinae	Obrini	<i>Obrium</i>	<i>Obrium</i> sp. NS-2015	15,680 KT945156
14 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Prioninae	Prionini	<i>Dorystenes</i>	<i>Dorystenes paradoxus</i>	15,922 NC_037927
15 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Prioninae	Callipogonini	<i>Callipogon</i>	<i>Callipogon relictus</i>	15,742 NC_037698
16 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lepturinae	Lepturini	<i>Anastrangalia</i>	<i>Anastrangalia sequensi</i>	16,269 NC_038090
17 Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Disteniinae		<i>Distenia</i>	<i>Distenia fimbriata</i>	9,776 JX221000
18 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae	Diabroticina	<i>Diabrotica</i>	<i>Diabrotica barberi</i>	16,366 NC_022935
19 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae	Diabroticina	<i>Diabrotica</i>	<i>Diabrotica virgifer</i>	16,650 KF658070
20 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae	Galerucines	<i>Galeruca</i>	<i>Galeruca daurica</i>	16,615 NC_027114
21 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae		<i>Paleosepharia</i>	<i>Paleosepharia posticata</i>	15,729 NC_033532
22 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae	Alticini	<i>Agasicles</i>	<i>Agasicles hygrophila</i>	15,917 NC_028332
23 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Cassidinae		<i>Rhadinosa</i>	<i>Rhadinosa nigrocyanea</i>	17,965 NC_037218
24 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Cassidinae		<i>Agonita</i>	<i>Agonita chinensis</i>	16,395 MF351622
25 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Chrysomelinae	Chrysomelini	<i>Goniocetena</i>	<i>Goniocetena intermedia</i>	18,064 MF563963
26 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Chrysomelinae	Chrysomelini	<i>Goniocetena</i>	<i>Goniocetena intermedia</i>	18,293 MF563962
27 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Chrysomelinae	Chrysomelini	<i>Gastrolina</i>	<i>Gastrolina thoracica</i>	16,109 NC_036108
28 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Bruchinae	Bruchini	<i>Acanthoscelides</i>	<i>Acanthoscelides obrectus</i>	16,130 NC_035677
29 Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Criocerinae		<i>Crioceris</i>	<i>Crioceris duodecimpunctata</i>	15,880 AF467886
30 Coleoptera	Cucujiformia	Chrysomeloidea	Vesperiidae	Philinae	Philini	<i>Spiniphilus</i>	<i>Spiniphilus spinicornis</i>	15,707 NC_029515

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No. Order	Suborder	Superfamily	Family	Subfamily	Tribes	Genus	Species	Species Accession no.
31	Coleoptera	Cucujiformia	Chrysomeloidea	Orsodacnidae		<i>Orsodacne</i>	<i>Orsodacne lineola</i>	9,828 JX220994
32	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae	<i>Stiophilus</i>	<i>Stiophilus zeamais</i>	18,517 KU757289
33	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae	<i>Stiophilus</i>	<i>Stiophilus oryzae</i>	17,602 KX373615
34	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae	<i>Stiophilus</i>	<i>Stiophilus zeamais</i>	18,105 KX373614
35	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae	<i>Rhynchophorus</i>	<i>Rhynchophorus ferrugineus</i>	15,924 KT428893
36	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae	<i>Sphenophorus</i>	<i>Sphenophorus</i> sp. BYU-CO246	15,052 GU176342
37	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae	<i>Cyrtotrachelus</i>	<i>Cyrtotrachelus buqueti</i>	15,035 NC_039705
38	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae	<i>Eucryptorhynchus</i>	<i>Eucryptorhynchus brandti</i>	15,597 KP455482
39	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae	<i>Eucryptorhynchus</i>	<i>Eucryptorhynchus scrobiculatus</i>	15,195 KP455510
40	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae	<i>Eucryptorhynchus</i>	<i>Eucryptorhynchus brandti</i>	15,122 NC_025945
41	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae	<i>Eucryptorhynchus</i>	<i>Eucryptorhynchus brandti</i>	16,919 KR088970
42	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae	<i>Eucryptorhynchus</i>	<i>Eucryptorhynchus scrobiculatus</i>	15,628 NC_026719
43	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Naupactini	<i>Naupactus</i>	<i>Naupactus xanthographus</i>	15,026 GU176345
44	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Tanymecini	<i>Symplezomias</i>	<i>Symplezomias velatus</i>	15,592 MF383367
45	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cyphicerini	<i>Myloцерinus</i>	<i>Myloцерinus aurolineatus</i>	17,762 NC_040931
46	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Polydrusini	<i>Pachyrhinus</i>	<i>Pachyrhinus yasumatsui</i>	16,472 MF807224
47	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Anthonomini	<i>Anthonomus</i>	<i>Anthonomus rubi</i>	17,476 NC_044714
48	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Anthonomini	<i>Anthonomus</i>	<i>Anthonomus rectirostris</i>	17,676 NC_044713
49	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Anthonomini	<i>Anthonomus</i>	<i>Anthonomus pomorum</i>	17,093 NC_044712
50	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Anthonomini	<i>Anthonomus</i>	<i>Anthonomus eugenii</i>	17,257 NC_044711
51	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Curculionini	<i>Curculio</i>	<i>Curculio davidi</i>	16,852 NC_034293
52	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Hylobiini	<i>Hylobiellus</i>	<i>Hylobiellus xiaoi</i>	16,123 NC_022680
53	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae		<i>Aegorhinus</i>	<i>Aegorhinus superciliosus</i>	15,121 NC_027577
54	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae		<i>Euplatypus</i>	<i>Euplatypus parallelus</i>	16,095 KX711704
55	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Alcidinae	<i>Alcidodes</i>	<i>Alcidodes juglans</i>	15,638 NC_041669
56	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Ceutorhynchinae	<i>Ceutorhynchus</i>	<i>Ceutorhynchus obstrictus</i>	20,124 NC_045101
57	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Scolytinae	<i>Scolytinae</i>	<i>Scolytinae</i> sp. BMNH 1274287	17,064 K1696206
58	Coleoptera	Cucujiformia	Curculionoidea	Rhynchitinae		<i>Cyllothynechites</i>	<i>Cyllothynechites ursulus</i>	14,508 MH156809
59	Coleoptera	Cucujiformia	Curculionoidea	Brentidae	Apioninae	<i>Leptidaption</i>	<i>Leptidaption squamigerum</i>	18,562 MN459662
60	Coleoptera	Cucujiformia	Tenebrionoidea	Tenebrionidae	Tenebrionidae	<i>Tribolium</i>	<i>Tribolium castaneum</i>	15,881 AJ312413

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No. Order	Suborder	Superfamily	Family	Subfamily	Tribes	Genus	Species	Species Accession no.
61	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrionidae incertae sedis	<i>Tribolium</i>	<i>Tribolium confusum</i>	15,813 KP420018
62	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrionidae incertae sedis	<i>Tribolium</i>	<i>Tribolium castaneum</i>	15,883 KM009121
63	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrionidae incertae sedis	<i>Tribolium</i>	<i>Tribolium andax</i>	15,925 NC_024600
64	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrionidae incertae sedis	<i>Tribolium</i>	<i>Tribolium castaneum</i>	15,881 NC_003081
65	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrionidae incertae sedis	<i>Tribolium</i>	<i>Tribolium castaneum</i>	15,877 KM244661
66	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrioninae	<i>Tenebrio</i>	<i>Tenebrio molitor</i>	15,785 NC_024633
67	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrioninae	<i>Tenebrio</i>	<i>Tenebrio obscurus</i>	15,771 NC_037196
68	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrioninae	<i>Tenebrio</i>	<i>Tenebrio molitor</i>	15,784 KP994554
69	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Tenebrioninae	<i>Zophobas</i>	<i>Zophobas atratus</i>	15,494 NC_041101
70	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Pimeliinae	<i>Asbolus</i>	<i>Asbolus verrucosus</i>	15,828 NC_027256
71	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Diaperinae	<i>Ulmoides</i>	<i>Ulmoides dermestoides</i>	15,434 NC_025332
72	Coleoptera	Cucujiformia	Tenebrionidea	Tenebrionidae	Lagrinae	<i>Adelum</i>	<i>Adelum</i> sp. NCS-2009	16,449 FJ613422
73	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Epicauta</i>	<i>Epicauta chinensis</i>	15,717 KP692789
74	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Epicauta</i>	<i>Epicauta aptera</i>	15,645 NC_031820
75	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Epicauta</i>	<i>Epicauta gorhami</i>	15,691 NC_036042
76	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Epicauta</i>	<i>Epicauta tibialis</i>	15,716 NC_036043
77	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Hycleus</i>	<i>Hycleus phaleratus</i>	16,003 NC_036045
78	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Hycleus</i>	<i>Hycleus chodschenicus</i>	16,257 KT808466
79	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Hycleus</i>	<i>Hycleus mureipoli</i>	15,923 NC_036044
80	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Mylabris</i>	<i>Mylabris aulica</i>	15,758 NC_036046
81	Coleoptera	Cucujiformia	Tenebrionidea	Meloidae	Meloinae	<i>Lytta</i>	<i>Lytta caraganae</i>	15,633 KX161859
82	Coleoptera	Cucujiformia	Tenebrionidea	Mordellidae	Mordellinae	<i>Mordella</i>	<i>Mordella atrata</i>	15,540 FJ859904
83	Coleoptera	Cucujiformia	Tenebrionidea	Coccinellidae	Coccinellinae	<i>Coccinella</i>	<i>Coccinella septempunctata</i>	18,965 JQ321839
84	Coleoptera	Cucujiformia	Tenebrionidea	Coccinellidae	Coccinellinae	<i>Coccinella</i>	<i>Coccinella ruga</i>	10,589 JX412767
85	Coleoptera	Cucujiformia	Tenebrionidea	Coccinellidae	Chilocorinae	<i>Chilocorus</i>	<i>Chilocorus bipustulatus</i>	12,229 MN053054
86	Coleoptera	Cucujiformia	Tenebrionidea	Coccinellidae	Coccinellinae	<i>Rodolia</i>	<i>Rodolia quadrinaculata</i>	12,660 MN053055
87	Coleoptera	Cucujiformia	Tenebrionidea	Coccinellidae	Coccinellinae	<i>Atolocarta</i>	<i>Atolocarta hexaspilota</i>	17,549 NC_042417
88	Coleoptera	Cucujiformia	Tenebrionidea	Coccinellidae	Coccinellinae	<i>Hammonia</i>	<i>Hammonia axyridis</i>	16,387 KR108208
89	Coleoptera	Cucujiformia	Tenebrionidea	Coccinellidae	Coccinellinae	<i>Halysia</i>	<i>Halysia sedecimguttata</i>	15,766 KT780652

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No.	Order	Suborder	Superfamily	Family	Subfamily	Tribes	Genus	Species	Species Accession no.
90	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Chelionemes</i>	<i>Chelionemes sexmaculata</i>	17,192 KM244706
91	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Propylea</i>	<i>Propylea japonica</i>	15,027 KM244660
92	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Halysini	<i>Halysini</i>	<i>Halysini</i> sp. HA	14,431 MG584728
93	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Coccinella</i>	<i>Coccinella transversoguttata</i>	15,806 MG584726
94	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Coelophora</i>	<i>Coelophora saucia</i>	11,942 MN053056
95	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Calvia</i>	<i>Calvia championorum</i>	17,575 KX132085
96	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Propylea</i>	<i>Propylea</i> sp. HSL-2016	15,915 KX132084
97	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Henosepilachna</i>	<i>Henosepilachna pusillanina</i>	16,216 NC_023469
98	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Henosepilachna</i>	<i>Henosepilachna vigintioctopunctata</i>	17,057 NC_041172
99	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Subcoccinella</i>	<i>Subcoccinella vigintiquatuor-punctata</i>	14,645 KT780695
100	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Afissula</i>	<i>Afissula</i> sp. XL-2019	15,339 MN053057
101	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	<i>Epilachna</i>	<i>Epilachna admirabilis</i>	17,445 MN053053
102	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Seymini	<i>Cryptolaemus</i>	<i>Cryptolaemus montiviverti</i>	10,914 KT878321
103	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae		<i>Unclassified Coccinellidae</i>	<i>Coccinellidae</i> sp. 1 EF-2015	16,631 KT780638
104	Coleoptera	Cucujiformia	Cucujoidea	Nitidulidae	Nitidulinae		<i>Aethina</i>	<i>Aethina tumida</i>	16,576 NC_036104
105	Coleoptera	Cucujiformia	Cucujoidea	Nitidulidae	Carpophilinae		<i>Carpophilus</i>	<i>Carpophilus dimidiatus</i>	15,717 MN604384
106	Coleoptera	Cucujiformia	Cucujoidea	Nitidulidae	Carpophilinae		<i>Carpophilus</i>	<i>Carpophilus pilosellus</i>	15,686 MN604383
107	Coleoptera	Cucujiformia	Cucujoidea	Laemophloeidae			<i>Cryptolestes</i>	<i>Cryptolestes pusillus</i>	15,502 NC_028204
108	Coleoptera	Cucujiformia	Cucujoidea	Laemophloeidae			<i>Cryptolestes</i>	<i>Cryptolestes ferrugineus</i>	15,511 NC_028203
109	Coleoptera	Cucujiformia	Cucujoidea	Laemophloeidae			<i>Cryptolestes</i>	<i>Cryptolestes tucicus</i>	15,517 KT070712
110	Coleoptera	Cucujiformia	Cucujoidea	Phloeostichidae			<i>Priastipha</i>	<i>Priastipha obscura</i>	16,603 EU877952
111	Coleoptera	Cucujiformia	Cucujoidea	Bothriideridae			<i>Dastarcus</i>	<i>Dastarcus helophoroides</i>	15,878 NC_024271
112	Coleoptera	Cucujiformia	Cucujoidea	Cucujidae			<i>Cucujus</i>	<i>Cucujus clavipes</i>	15,642 GU176341
113	Coleoptera	Cucujiformia	Cucujoidea	Erotylidae			<i>Unclassified Erotylidae</i>	<i>Erotylidae</i> sp. BMNH 1274392	16,553 KT696227
114	Coleoptera	Cucujiformia	Clerioidea	Cleridae	Clerinae		<i>Trichodes</i>	<i>Trichodes siniae</i>	16,047 NC_033340
115	Coleoptera	Cucujiformia	Clerioidea	Chaetosomatidae			<i>Chaetosoma</i>	<i>Chaetosoma scarioides</i>	15,511 NC_011324
116	Coleoptera	Cucujiformia	Clerioidea	Prionoceridae			<i>Ildgia</i>	<i>Ildgia oculata</i>	15,805 NC_044896
117	Coleoptera	Cucujiformia	Lymexyloidea	Lymexylinidae	Melittomminae		<i>Melittomma</i>	<i>Melittomma</i> sp. MEL01	9,674 JX412776
118	Coleoptera	Scarabaeiformia	Scarabaeoidea	Lucanidae	Lucaninae		<i>Prosopocoilus</i>	<i>Prosopocoilus gracilis</i>	16,736 KP755805
119	Coleoptera	Scarabaeiformia	Scarabaeoidea	Scarabaeidae	Melolonthinae		<i>Cheirotonus</i>	<i>Cheirotonus jansoni</i>	17,249 NC_023246
120	Coleoptera	Staphyliniformia	Staphylinioidea	Staphylinidae	Staphylininae		<i>Necrophila</i>	<i>Necrophila americana</i>	16,902 NC_018352

Supplementary Table S2. Nucleotide composition and skews rate in the forward strand of *Anoplophora horsfieldi* mitochondrial DNA by regions.

Gene/re- gion	Nucleotide frequency (%)				A+T	AT- skew	GC- skew
	T	C	A	G			
Total	39.57	12.47	39.74	8.22	79.31	0.002	-0.206
L-strand	44.45	9.54	37.40	8.61	81.85	-0.086	-0.052
H-strand	42.30	12.88	34.32	10.50	76.62	-0.104	-0.102
tRNA	38.07	12.18	40.78	8.98	78.84	0.034	-0.151
rRNA	40.03	13.25	40.85	5.88	80.88	0.010	-0.385
PCGs	44.79	10.74	33.50	10.96	78.30	-0.144	0.010
1 st	36.97	11.26	34.33	17.43	71.30	-0.037	0.215
2 nd	47.59	17.54	21.05	13.82	68.63	-0.387	-0.119
3 rd	49.82	3.42	45.14	1.62	94.96	-0.049	-0.358
ATP6	42.96	13.63	34.81	8.59	77.78	-0.105	-0.227
ATP8	49.36	9.62	38.46	2.56	87.82	-0.124	-0.579
COX1	39.99	14.84	30.40	14.78	70.38	-0.136	-0.002
COX2	40.70	14.53	33.72	11.05	74.42	-0.094	-0.136
COX3	40.68	13.31	32.70	13.31	73.38	-0.109	0.000
ND1	46.73	7.17	32.17	13.92	78.90	-0.184	0.320
ND2	47.18	10.39	35.41	7.02	82.59	-0.143	-0.193
ND3	48.86	9.94	33.81	7.39	82.67	-0.182	-0.148
ND4	49.32	7.07	32.56	11.05	81.88	-0.205	0.220
ND4L	50.35	6.25	33.33	10.07	83.68	-0.203	0.234
ND5	46.44	6.88	35.18	11.49	81.62	-0.138	0.251
ND6	47.02	9.72	38.49	4.76	85.52	-0.100	-0.342
Cytb	42.19	14.74	32.28	10.79	74.47	-0.133	-0.155
12S rRNA	38.56	13.68	41.54	6.22	80.10	0.037	-0.375
16S rRNA	40.93	12.96	40.46	5.66	81.38	-0.006	-0.392
Control region	42.52	10.50	43.92	3.06	86.44	0.016	-0.548
Overall	43.90	11.14	35.85	9.11	79.75	-0.103	-0.129

AT skew = (A% - T%)/(A% + T%); GC skew = (G% - C%)/(G% + C%).

Supplementary Table S3. Nucleotide composition and skews rate in the forward strand of *Anoplophora chinensis* mitochondrial DNA by regions.

Gene/re- gion	Nucleotide frequency (%)				A+T	AT skew	GC skew
	T	C	A	G			
Total	38.17	13.57	39.48	8.78	77.65	0.017	-0.214
L-strand	44.65	6.66	35.80	12.89	80.45	-0.110	0.318
H-strand	40.55	14.35	34.09	11.01	74.64	-0.087	-0.132
tRNA	37.28	10.17	40.04	12.52	77.32	0.036	0.104
rRNA	40.99	6.17	39.02	13.82	80.01	-0.025	0.383
PCGS	43.51	11.82	32.84	11.82	76.35	-0.140	0.000
1 st	36.01	12.43	33.37	18.19	69.38	-0.038	0.188
2 nd	47.36	17.65	20.92	14.07	68.27	-0.387	-0.113
3 rd	47.17	5.39	44.23	3.21	91.40	-0.032	-0.254
ATP6	40.15	15.85	35.11	8.89	75.26	-0.067	-0.281
ATP8	44.87	10.90	41.03	3.21	85.90	-0.045	-0.545
COX1	37.33	15.94	31.37	15.36	68.70	-0.087	-0.019
COX2	40.55	15.26	32.99	11.19	73.55	-0.103	-0.154
COX3	39.80	15.21	31.94	13.05	71.74	-0.110	-0.076
ND1	47.15	7.49	31.01	14.35	78.16	-0.206	0.314
ND2	43.72	14.44	33.43	8.41	77.15	-0.133	-0.264
ND3	47.16	12.50	31.82	8.52	78.98	-0.194	-0.189
ND4	48.80	6.84	31.43	12.93	80.23	-0.216	0.308
ND4L	50.00	6.94	30.90	12.15	80.90	-0.236	0.273
ND5	45.88	7.48	33.78	12.86	79.66	-0.152	0.264
ND6	46.03	9.33	38.89	5.75	84.92	-0.084	-0.237
Cytb	41.49	15.26	32.11	11.14	73.60	-0.128	-0.156
12S rRNA	40.55	6.97	37.69	14.80	78.23	-0.037	0.360
16S rRNA	41.32	5.66	39.83	13.20	81.15	-0.018	0.400
Control region	43.77	5.22	41.82	9.20	85.59	-0.023	0.276
Overall	42.97	10.78	35.00	11.25	77.97	-0.104	0.022

AT skew = (A% - T%)/(A% + T%); GC skew = (G% - C%)/(G% + C%).

Supplementary Table S4. Nucleotide composition and skews rate in the forward strand of *Anoplophora glabripennis* mitochondrial DNA by regions.

Gene/re- gion	Nucleotide frequency (%)				A+T	AT skew	GC skew
	T	C	A	G			
Total	38.71	13.07	39.62	8.59	78.34	0.012	-0.207
L-strand	44.69	6.99	35.10	13.23	79.79	-0.120	0.309
H-strand	41.50	13.04	35.57	9.88	77.07	-0.077	-0.138
tRNA	38.09	9.85	39.97	12.09	78.06	0.024	0.102
rRNA	40.85	6.23	38.94	13.98	79.79	-0.024	0.383
PCGS	43.88	11.47	33.14	11.51	77.02	-0.139	0.002
1 st	36.13	12.09	34.13	17.66	70.25	-0.028	0.187
2 nd	47.38	17.74	21.12	13.76	68.50	-0.383	-0.126
3 rd	48.13	4.57	44.18	3.11	92.32	-0.043	-0.190
ATP6	43.01	13.24	34.52	9.23	77.53	-0.109	-0.179
ATP8	46.15	10.26	41.03	2.56	87.18	-0.059	-0.600
COX1	38.24	15.17	31.50	15.10	69.73	-0.097	-0.002
COX2	40.26	15.26	33.58	10.90	73.84	-0.091	-0.167
COX3	40.46	14.76	31.17	13.61	71.63	-0.130	-0.040
ND1	46.23	7.54	31.03	15.19	77.26	-0.197	0.336
ND2	43.13	14.14	34.92	7.81	78.04	-0.105	-0.288
ND3	49.28	10.60	32.38	7.74	81.66	-0.207	-0.156
ND4	49.02	7.44	31.28	12.26	80.30	-0.221	0.244
ND4L	52.26	6.27	31.71	9.76	83.97	-0.245	0.217
ND5	45.39	7.48	34.99	12.15	80.37	-0.129	0.238
ND6	45.88	9.86	39.03	5.23	84.91	-0.081	-0.307
Cytb	42.46	14.74	31.93	10.88	74.39	-0.142	-0.151
12S rRNA	40.69	6.70	37.34	15.26	78.04	-0.043	0.390
16S rRNA	40.89	5.91	40.00	13.20	80.89	-0.011	0.381
Control region	42.87	8.25	45.11	3.77	87.98	0.025	-0.373
Overall	43.42	10.51	35.33	10.74	78.75	-0.105	-0.005

AT skew = (A% - T%) / (A% + T%); GC skew = (G% - C%) / (G% + C%).