Pakistan J. Zool., pp 1-19, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220824100800

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

Haifen Qin[1,2], Yujia Liu[1], Yujie Zhang[1], Jingfeng Liu[1], Zhenkun Zhao[1,2] and Lichun Jiang[1,2]*

[1]Key Laboratory for Molecular Biology and Biopharmaceutics, School of Life Science and Technology, Mianyang Normal University, Mianyang, Sichuan, 621000, P.R. China.
[2]Ecological Security and Protection Key Laboratory of Sichuan Province, Mianyang Normal University, Mianyang, Sichuan, 621000, P.R. China.

Article Information
Received 24 August 2022
Revised 15 September 2022
Accepted 01 October 2022
Available online 08 May 2023
(early access)

Authors' Contribution
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**

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.

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,

* Corresponding author: jiang_lichun@126.com
0030-9923/2023/0001-0001 $ 9.00/0

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.ccbb.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 jansoni* (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 v.0.1.1 (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 obstrictus* (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 I. The mtDNA gene arrangement of three *Anoplophora* species (*Anoplophora horsfieldi*, *Anoplophora chinensis*, *Anoplophora glabripennis*).

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

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

* T, 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).

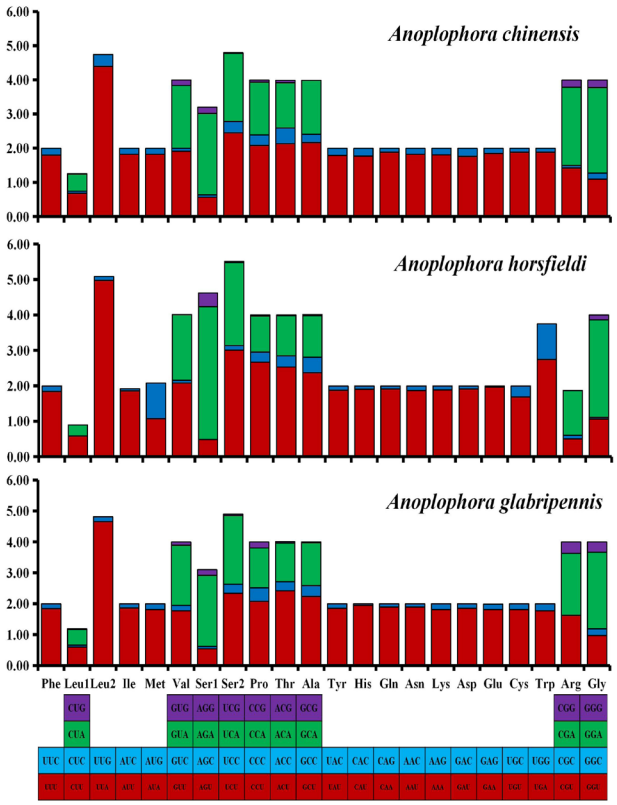


Fig. 2. The relative synonymous codon usage (RSCU) in the mitogenomes of three *Anoplophora* species. Codon families are provided on the x-axis along with the different combinations of synonymous codons that code for that amino acid. RSCU are provided on the y-axis.

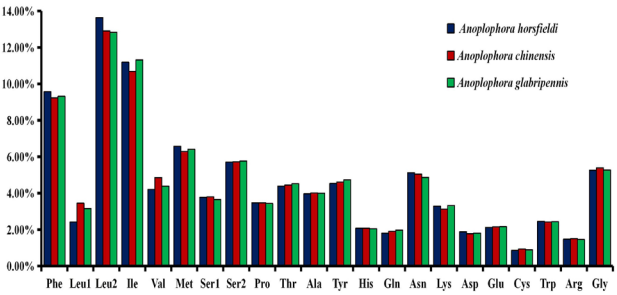


Fig. 3. Total codons in three *Anoplophora* mitogenomes.

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
Anoplophora horsfieldi												
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
1st	3711	1274	1372	647	418	36.97	11.26	34.33	17.43	71.30	-0.037	0.215
2nd	3711	781	1766	513	651	47.59	17.54	21.05	13.82	68.63	-0.387	-0.119
3th	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
1st	2591	925	864	442	360	33.35	13.89	35.70	17.06	69.05	0.034	0.102
2nd	2591	611	1157	331	492	44.65	18.99	23.58	12.77	68.24	-0.309	-0.196
3th	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
1st	2672	982	1142	301	247	42.74	9.24	36.75	11.26	79.49	-0.075	0.099
2nd	2672	829	1210	276	357	45.28	13.36	31.03	10.33	76.31	-0.187	-0.128
3th	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
Anoplophora chinensis												
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
1st	3710	1238	1336	675	461	36.01	12.43	33.37	18.19	69.38	-0.038	0.188
2nd	3710	776	1757	522	655	47.36	17.65	20.92	14.07	68.27	-0.387	-0.113
3th	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
1st	2595	900	834	463	398	32.14	15.34	34.68	17.84	66.82	0.038	0.075
2nd	2595	611	1163	331	490	44.82	18.88	23.55	12.76	68.36	-0.311	-0.194
3th	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
1st	2666	966	1115	413	172	44.65	6.66	35.80	12.89	80.45	-0.110	0.318
2nd	2666	785	1213	386	282	45.50	10.58	29.44	14.48	74.94	-0.214	0.156
3th	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
Anoplophora glabripennis												
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
1st	3698	1262	1336	653	447	36.13	12.09	34.13	17.66	70.25	-0.028	0.187
2nd	3698	781	1752	509	656	47.38	17.74	21.12	13.76	68.50	-0.383	-0.126
3th	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
1st	2954	1076	1004	451	423	33.99	14.32	36.43	15.27	70.41	0.035	0.032
2nd	2955	776	1328	341	510	44.94	17.26	26.26	11.54	71.20	-0.262	-0.199
3th	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
1st	2271	793	967	364	147	42.58	6.47	34.92	16.03	77.50	-0.099	0.425
2nd	2271	635	1049	325	262	46.19	11.54	27.96	14.31	74.15	-0.246	0.107
3th	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.

Amino acid	Codon	Count			RSCU			Frequency (%)			Amino 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).

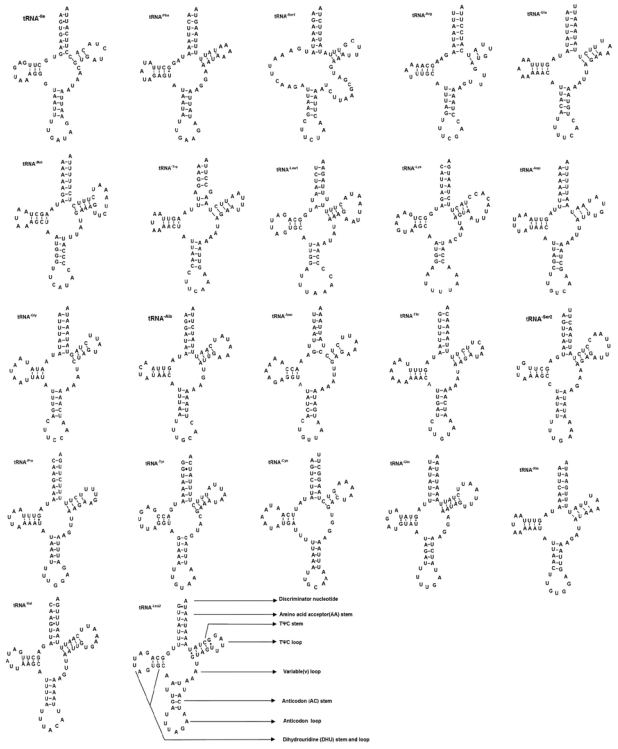


Fig. 4. Putative tRNA secondary structures predicted from the 22 tRNA gene sequences found in the *Anoplophora horsfieldi* mitogenome.

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

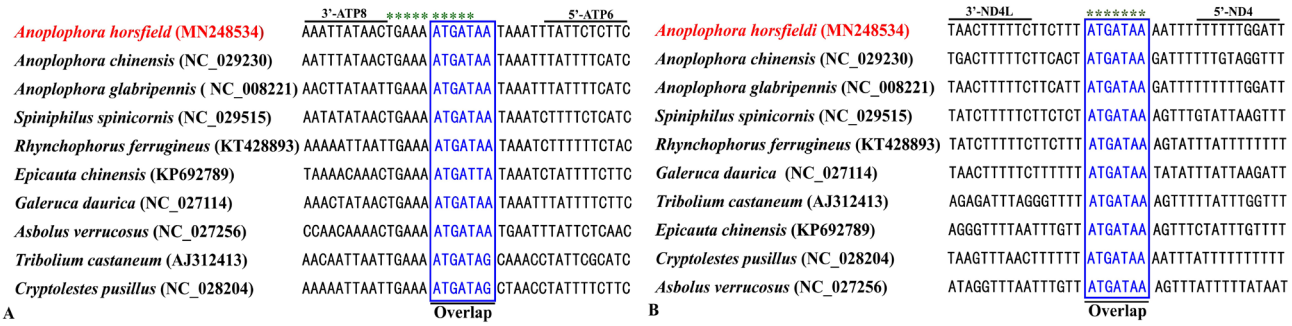


Fig. 5. Alignment of overlapping region across Cucujiformia and other insects. (A) Atp8 and Atp6, the motifs "ATGATA/TA/G" in the box are conservative; (B) ND4L and ND4, the motifs "ATGATAA" in the box are conservative.

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 "ATGTTAA" was found in the other three suborders' mitogenomes (Adephaga, Archostemata and Myxophaga) (Wang and Tang, 2018).

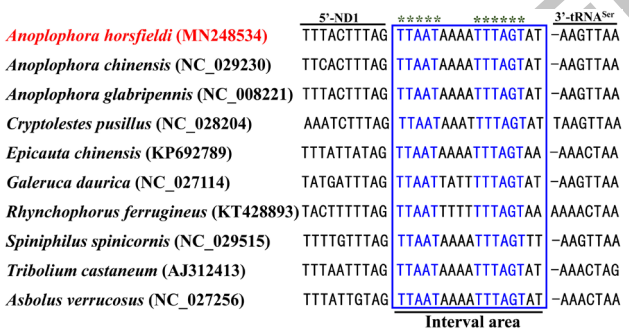


Fig. 6. Alignment of the intergenic spacer region between trnS (UCN) and ND1 of several Cucujiformia insects. The shaded "TTAAT" and "TTTAGT" motif is conserved across the Cucujiformia species.

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* specie, three conserved sequence blocks (CSBs) were recognized in those species. These CBSs 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).

***rrn*S-14,654-AAAAAAAAGAATACTCCCCCCTATTAAAAATATTCAAAACCTCATTTTTTAGCACTAA**
AACTTTCTATATAAAATTATTTCAATATCCCCTAAATACTGATTTTCATAAATACTTCCATAAAAC
TCATTTATAAAATTATTTCAATATCCCCTAAATACTGATTTTCATAAATACTTCCATAAAACTCAT
TTATAAAATTATTTCAATATCCCCTAAATACTGATTTTCATAAATACTTCCATAAAACTCATTTAT
AAAATTATTTCAATATCCCCTAAATACTGATTTTCATAAATACTTCCATAAAACTCATTTATAAAA
TTATTTCAATATCCCCTAAATACTGATTTTCATAAATACTTCCATAAAACTCATTTATAAAATTAT
TTCAATATCCCCTAAATACTGATTTTCATAAATACTTCCATTTAAATATAATCSB1**AATAATTTTATAAA**
CATATTATTATATAAAAATTTAATATTTTATATAATTAAAATAATTATTTAATAAAATTATAATTTT**
ATATTATATCATTTTTTATACTAATATAAAAAAAAGGGTAGTTTTTTTTTTTTTTTTAAAAATTGTT
ATTTTCAATAACAAATTTATATATTAATAATATATAAATTATTTATATATATATAACAATTATATTT
ATATATATTACTAAATATATTATATATATATATAAGTATCSB2**ATATTATTTTTATATTTATATTAAATTGTT**
ATATATATATAAGATATTTATATATCTATAATAATATAATATAAATGTATATATATAAATAATAAA
ATAAATAATATTGATTCATTATAGAAATAATTAATACTAATATAAATTTCTATTATCAATTTTATTT**
TTTTCATTATTGATTTATTTAAAATAGTATTATTCACTAAAATTTTATTAATATCTGATAAAAATA
GGAGATCCTTATATTAATATAAATAGCTTATATAAATATATAAACTGATTAGGCATATATTAATAT
AATTTTTATTTTAACAAAAAATAAAAAAAAAAAAAAATGTAAAAAAAATACAATCATTGCATTTTCT**
TTTAGTTAATTTTTCSB3**TTTCGCTAAAACCCAATTTTTACAAAAATTTACATTAAATTCATGTTTATTTA**
ATTTAATAACTAAAAAAAGTTAT-15,796-*trn*I

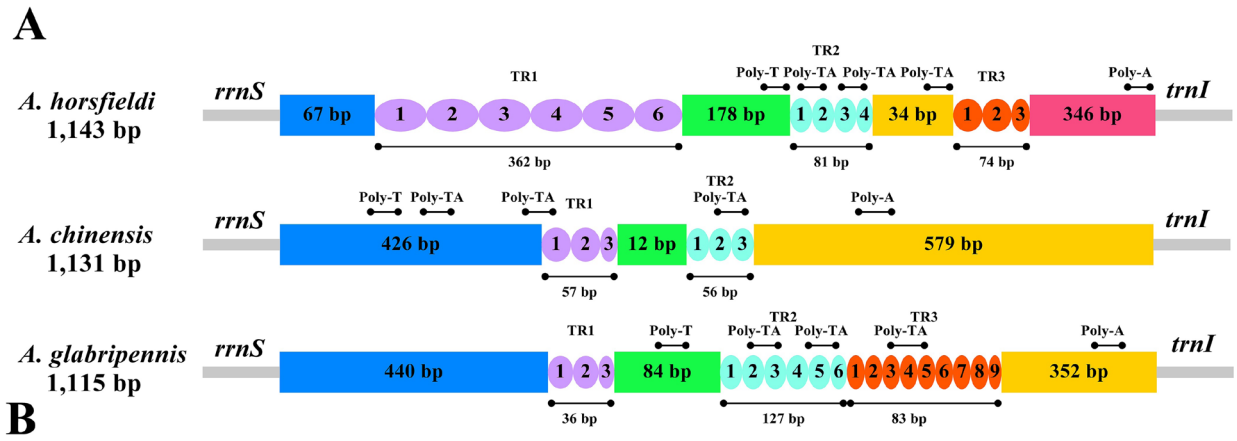


Fig. 7. (A) Organization of the A+T-rich regions in the *Anoplophora horsfieldi* mitogenomes. The square box represents the feature sequence CSB1-3. The poly-T stretch is underlined while the poly-A stretch is double underlined. The single microsatellite T/A repeats sequence are indicated by single underlining. (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 *Psacothea* 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

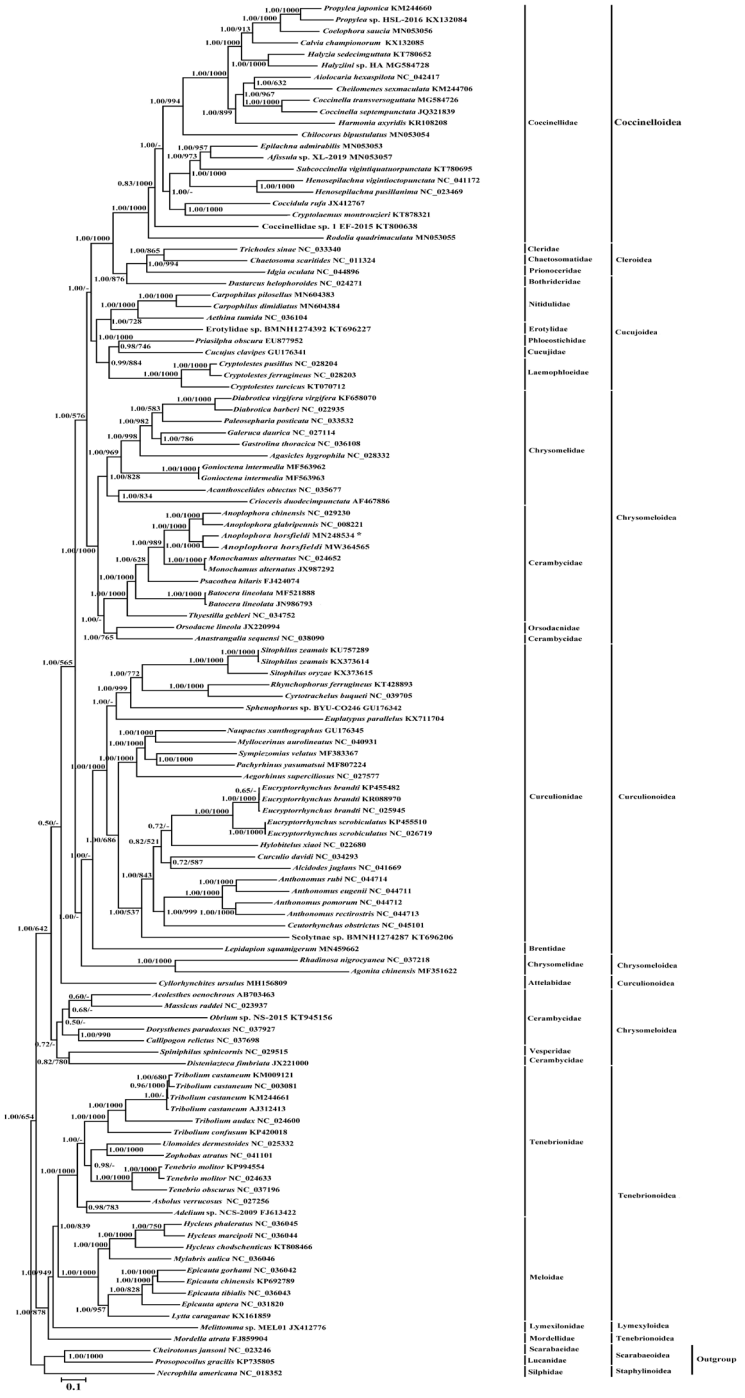


Fig. 8. Phylogenetic tree of the relationships among 117 mitogenome sequences of Cucujiformia based on the nucleotide dataset of the 13 mitochondrial protein-coding genes of 11,154 nucleotides with *Prosopocoilus gracilis* (Coleoptera: Scarabaeiformia: Lucanidae: KP735805), *Cheirotonus jansoni* (Coleoptera: 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 helps people to further the studies of molecular evolution, and Cucujiformia mitogenome architecture and phylogenetics.

ACKNOWLEDGEMENT

We would like to express our gratitude to all those who helped us during the writing of this manuscript.

Funding

This research was supported by the Natural Science Foundation Project of Science and Technology, Department of Sichuan Probince (No. 2023NSFSC0206), the National Natural Science Foundation of China (No. 32270457), the Research Project of Ecological Security and Protection Key Laboratory of Sichuan Province (No. ESP2003).

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

Sattement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Andrews, R., Kubacka, M.I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M. and Howell, N., 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat. Genet.,* **23**: 147. https://doi.org/10.1038/13779

Beckenbach, A.T., 2011. Mitochondrial genome sequences of representatives of three families of scorpionflies (Order Mecoptera) and evolution in a major duplication of coding sequence. *Genome,* **54:** 368-376. https://doi.org/10.1139/g11-006

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

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., Pütz, J., Middendorf, M. and Stadler, P.F., 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.,* **69:** 313-319. https://doi.org/10.1016/j.ympev.2012.08.023

Bocak, C., Barton, L., Crampton-Platt, A., Chesters, D., Ahrens, D. and Vogler, A.P., 2014. Building the coleoptera tree-of-life for > 8000 species: Composition of public dna data and fit with linnaean classification. *Syst. Ent.,* **39:** 97-110. https://doi.org/10.1111/syen.12037

Bouchard, P., Bousquet, Y., Davies, A.E., Alonso-Zarazaga, M.A., Lawrence, J.F., Lyal, C.H.C.,

Newton, A., Reid, C., Schmitt, M. and Slipinski, A., 2011. Family-group names in Coleoptera (Insecta). *Zookeys,* **88:** 1-972. https://doi.org/10.3897/zookeys.88.807

Bouchard, P., Grebennikov, V.V., Smith, A.B.T. and Douglas, H., 2009. Chapter 11. Biodiversity of coleoptera. In: *Insect biodiversity: Science and society* (eds. R.G. Foottit and P.H. Adler). Blackwell. Publishing. Ltd., Oxford

Cabrera-Brandt, M.A. and Gaitan-Espitia, J.D., 2015. Phylogenetic analysis of the complete mitogenome sequence of the raspberry weevil, *Aegorhinus superciliosus* (Coleoptera: Curculionidae), supports monophyly of the tribe Aterpini. *Gene,* **571:** 205-211. https://doi.org/10.1016/j.gene.2015.06.059

Cameron, S.L. and Whiting, M.F., 2008. The complete mitochondrial genome of the tobacco hornworm *Manduca sexta* (Insecta: Lepidoptera: Sphingidae) and an examination of mitochondrial gene variability within butterflies and moths. *Gene,* **40:** 112-123. https://doi.org/10.1016/j.gene.2007.10.023

Cameron, S.L., 2014. Insect mitochondrial genomics: Implications for evolution and phylogeny. *Annu. Rev. Ent.,* **59:** 95-117. https://doi.org/10.1146/annurev-ento-011613-162007

Cao, J., Wang, Y., and Li, W., 2019. Comparative mitogenomic analysis of species in the subfamily Amphinemurinae (Plecoptera: Nemouridae) reveal conserved mitochondrial genome organization. *Int. J. Biol. Macromol.,* **138:** 292-301. https://doi.org/10.1016/j.ijbiomac.2019.07.087

Chen, S., Xie, Y. and Deng, G., 1959. *Economic entomology of China: Volume 1. Coleoptera: Cerambycidae*. Science Press. Beijing, China.

Chen, Z.T. and Du, Y.Z., 2015. Comparison of the complete mitochondrial genome of the stonefly *Sweltsa longistyla* (Plecoptera: Chloroperlidae) with mitogenomes of three other stoneflies. *Gene,* **558:** 82–87. https://doi.org/10.1016/j.gene.2014.12.049

Chen, Z.T. and Du, Y.Z., 2017. First mitochondrial genome from Nemouridae (Plecoptera) reveals novel features of the elongated control region and phylogenetic implications. *Int. J. mol. Sci.,* **18:** 996. https://doi.org/10.3390/ijms18050996

Christoph, H., Lutz, B. and Bastien, C., 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads-a baiting and iterative mapping approach. *Nucl. Acids Res.,* **41:** e129. https://doi.org/10.1093/nar/gkt371

Coates, B.S., 2014. Assembly and annotation of full mitochondrial genomes for the corn rootworm species, *Diabrotica virgifera virgifera* and *Diabrotica barberi* (Insecta: Coleoptera: Chrysomelidae), using Next Generation Sequence data. *Gene,* **542:** 190-197. https://doi.org/10.1016/j.gene.2014.03.035

Crampton-Platt, A., Timmermans, M.J.T.N., Gimmel, M.L., Kutty, S.N., Cockerill, T.D., Vun Khen C. and Vogler, A.P., 2015. Soup to tree: the phylogeny of beetles inferred by mitochondrial metagenomics of a Bornean rainforest sample. *Mol. Biol. Evol.,* **32:** 2302-2316. https://doi.org/10.1093/molbev/msv111

Crowson, R.A., 1960. The phylogeny of coleopteran. *Annu. Rev. Ent.,* **5**: 111-134. https://doi.org/10.1146/annurev.en.05.010160.000551

De Rijk, P., Wuyts, J. and De Wachter, R., 2003. RnaViz 2: An improved representation of RNA secondary structure. *Bioinformatics,* **19:** 99-300. https://doi.org/10.1093/bioinformatics/19.2.299

Dierckxsens, N., and Mardulyn, P. and Smits, G., 2017. NOVOPlasty: De novo assembly of organelle genomes from whole genome data. *Nucl. Acids Res.,* **45:** e18. https://doi.org/10.1093/nar/gkw955

Dowton, M., Castro, L.R. and Austin, A.D., 2002. Mitochondrial gene rearrangements as phylogenetic characters in the invertebrates: The examination of genome morphology. *Invertebr. Syst.,* **16:** 345-356. https://doi.org/10.1071/IS02003

Du, C., He, S.L., Song, X.H., Liao, Q., Zhang, X.Y. and Yue, B.S., 2016. The complete mitochondrial genome of *Epicauta chinensis* (Coleoptera: Meloidae) and phylogenetic analysis among coleopteran insects. *Gene,* **578:** 274-280. https://doi.org/10.1016/j.gene.2015.12.036

Du, Y.M., Zhang, C.N., Dietrich, C.H., Zhang, Y.L. and Dai, W., 2017. Characterization of the complete mitochondrial genomes of *Maiestas dorsalis* and *Japananus hyalinus* (Hemiptera: Cicadellidae) and comparison with other Membracoidea. *Sci. Rep.* U.K., **7:** 14197. https://doi.org/10.1038/s41598-017-14703-3

Fang, J., Qian, L., Xu, M., Yang, X., Wang, B. and An, Y., 2016. The complete nucleotide sequence of the mitochondrial genome of the Asian longhorn beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Mitochondrial DNA A,* **27:** 3299-3300. https://doi.org/10.3109/19401736.2015.1015012

Farrell, B.D. and Sequeira, A.S., 2004. Evolutionary rates in the adaptive radiation of beetles on plants. *Evolution,* **58:** 1984-2001. https://doi.org/10.1111/j.0014-3820.2004.tb00484.x

Friedrich, M. and Muqim, N., 2003. Sequence and phylogenetic analysis of the complete mitochondrial genome of the flour beetle *Tribolium castaneum*. *Mol. Phylogenet. Evol.,* **26:** 502-512. https://doi.org/10.1016/S1055-7903(02)00335-4

Gómez-Zurita, J., Hunt, T., Kopliku, F. and Vogler, A.P., 2007. Recalibrated tree of leaf beetles (Chrysomelidae) indicates independent diversification of angiosperms and their insect herbivores. *PLoS One,* **2:** e360. https://doi.org/10.1371/journal.pone.0000360

Grimaldi, D. and Engel, M.S., 2003. Evolution of the insects, Mitochondrial genome of the flour beetle *Tribolium castaneum*. *Mol. Phylogenet. Evol.,* **26:** 502-512. https://doi.org/10.1016/S1055-7903(02)00335-4

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol.,* **59:** 307-321. https://doi.org/10.1093/sysbio/syq010

Hao, J.S., Sun, Q.Q., Zhao, H.B., Sun, X.Y., Gai, Y.H. and Yang, Q., 2012. The complete mitochondrial genome of *Ctenoptilum vasava* (Lepidoptera: Hesperiidae: Pyrginae) and its phylogenetic implication. *Int. J. Geno.,* **2012:** 328049. https://doi.org/10.1155/2012/328049

Huang, M.C., Wang, Y.Y., Liu, X.Y., Li, W., Kang, Z., Wang, K., Li, X. and Yang, D., 2015. The complete mitochondrial genome and its remarkable secondary structure for a stonefly *Acroneuria hainana* Wu (Insecta: Plecoptera, Perlidae). *Gene,* **557:** 52-60. https://doi.org/10.1016/j.gene.2014.12.009

Huang, Y., Liu, Y., Zhu, X.Y., Xin, Z.Z., Zhang, H.B., Zhang, D.Z., Wang, J.L., Tang, B.P., Zhou, C.L., Liu, Q.N. and Dai, L.S., 2019. Comparative mitochondrial genome analysis of *Grammodes geometrica* and other noctuid insects reveals conserved mitochondrial genome organization and phylogeny. *Int. J. Biol. Macromol.,* **125:** 1257-1265. https://doi.org/10.1016/j.ijbiomac.2018.09.104

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O.S., Wild, R., Hammond, P.M., Ahrens, D., Balke, M. and Caterino, M.S., 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science,* **318:** 1913-1916. https://doi.org/10.1126/science.1146954

James, B.S. and Andrew, T.B., 2006. Insect mitochondrial genomics 2: The complete mitochondrial genome sequence of a giant stonefly, *Pteronarcys princeps*, asymmetric direcional mutation bias, and conserved plecopteran A + T-region elements. *Genome,* **49:** 815-824. https://doi.org/10.1139/g06-037

Jin, S.B., Kim, I., Sohn, H.D. and Jin, B.R., 2004. The mitochondrial genome of the firefly, Pyrocoelia rufa: complete DNA sequence, genome organization and phylogenetic analysis with other insects. *Mol. Biol. Evol.,* **32:** 978-985. https://doi.org/10.1016/j.ympev.2004.03.009

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

Kim, I., Lee, E.M., Seol, K.Y., Yun, E.Y., Lee, Y.B., Hwang, J.S. and Jin, B.R., 2006. The mitochondrial genome of the Korean hairstreak, *Coreana raphaelis* (Lepidoptera: Lycaenidae). *Insect mol. Biol.,* **15:** 217-225. https://doi.org/10.1111/j.1365-2583.2006.00630.x

Kim, K.G., Hong, M.Y., Kim, M.J., Im, H.H., Kim, M.I., Bae, C.H., Seo, S.J., Lee, S.H. and Kim, I., 2009. Complete mitochondrial genome sequence of the yellow-spotted long-horned beetle *Psacothea hilaris* (Coleoptera: Cerambycidae) and phylogenetic analysis among coleopteran insects. *Mol. Cells,* **27:** 429-441. https://doi.org/10.1007/s10059-009-0064-5

Kumar, S., Stecher, G. and Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.,* **33:** 870-1874. https://doi.org/10.1093/molbev/msw054

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics,* **23:** 2947-2948. https://doi.org/10.1093/bioinformatics/btm404

Lawrence, J.F., Slipinski, A., Seago, A.E., Thayer, M.K., Newton, A.F. and Marvaldi, A.E., 2011. Phylogeny of the Coleoptera based on morphological characters of adults and larvae. *Annls Zool.,* **61:** 1-217. https://doi.org/10.3161/000345411X576725

Li, H., Liu, H., Shi, A., Stys, P., Zhou, X. and Cai, W., 2012. The complete mitochondrial genome and novel gene arrangement of the unique-headed bug *Stenopirates* sp. (Hemiptera: Enicocephalidae). *PLoS One,* **7:** e29419. https://doi.org/10.1371/

journal.pone.0029419

Li, L., Liu, G., Sun, T., Xin, T., Li, M., Zou, Z. and Xia, B., 2016. Complete mitochondrial genome of *Cryptolestes pusillus* (Coleoptera: Laemophloeidae). *Mitochondrial DNA A,* **27:** 3703-3704. https://doi.org/10.3109/19401736.2015.1079865

Li, N., Hu, G. and Hua, B., 2019. Complete mitochondrial genomes of *Bittacus strigosus* and *Panorpa debilis* and genomic comparisons of Mecoptera. *Int. J. Biol. Macromol.,* **140:** 672-681. https://doi.org/10.1016/j.ijbiomac.2019.08.152

Li, Q., Wei, S.J., Shi, M. and Chen, X.X., 2015. The complete mitochondrial genome of *Neopanorpa pulchra* (Mecoptera: Panorpidae). *Mitochondrial DNA A,* **26:** 305-306. https://doi.org/10.3109/19401736.2013.825787

Li, W., Yang, X., Qian, L., An, Y. and Fang, J., 2016. The complete mitochondrial genome of the citrus long-horned beetle, *Anoplophora chinensis* (Coleoptera: Cerambycidae). *Mitochondrial DNA A*, **27:** 4665-4667. https://doi.org/10.3109/19401736.2015.1106493

Li, X., Ou, J., Wei, Z., Li, Y. and Tian, Y., 2016. The mitogenomes of three beetles (Coleoptera: Polyphaga: Cucujiformia): New gene rearrangement and phylogeny. *Biochem. Syst. Ecol.,* **69:** 101-107. https://doi.org/10.1016/j.bse.2016.08.012

Liu, J., Jia, P., Luo, T. and Wang, Q., 2017. Complete mitochondrial genome of white-striped long-horned beetle, *Batocera lineolata* (Coleoptera: Cerambycidae) by next-generation sequencing and its phylogenetic relationship within superfamily Chrysomeloidea. *Mitochondrial DNA B,* **2:** 520-521. https://doi.org/10.1080/23802359.2017.1361797

Liu, Y.Q., Chen, D.B., Liu, H.H., Hu, H.L., Bian, H.X., Zhang, R.S., Yang, R.S., Jiang, X.F. and Shi, S.L., 2018. The complete mitochondrial genome of the longhorn beetle *Dorysthenes paradoxus* (Coleoptera: Cerambycidae: Prionini) and the implication for the phylogenetic relationships of the Cerambycidae species. *J. Insect Sci.,* **18:** 21. https://doi.org/10.1093/jisesa/iey012

Lowe, T.M. and Chan, P.P., 2016. tRNAscan-SE On-line: Integrating search and context for analysis of transfer RNA genes. *Nucl. Acids Res.,* **44:** W54-W57. https://doi.org/10.1093/nar/gkw413

Lu, H.F., Su, T.J., Luo, A.R., Zhu, C.D. and Wu, C.S., 2013. Characterization of the complete mitochondrion genome of diurnal moth *Amata emma* (Butler) (Lepidoptera: Erebidae) and its phylogenetic implications. *PLoS One,* **8:** e72410. https://doi.org/10.1371/journal.pone.0072410

Luo, R., Liu, B, Xie, Y., Li, Z.Y., Huang, W.H., Yuan, J.Y., He, G.Z., Chen, Y.X., Pan, Q., Liu, Y.J., Tang, J.B., Wu, G.X., Zhang, H., Shi, Y.J., Liu, Y., Yu, C., Wang, B., Lu, Y., Han, C.L., Cheung, D.W., Yiu, S.M., Peng, S.L., Zhu, X.Q., Liu, G.M., Liao, X.K., Li, Y.R., Yang, H.M., Wang, J., Lam T.W. and Wang, J., 2012. SOAPdenovo2: An empirically improved memory-efficient short-read de novo assembler. *Giga Sci.,* **1:** 18. https://doi.org/10.1186/2047-217X-1-18

Ma, C., Yang, P.C., Jiang, F., Chapuis, M.P., Shali, Y., Sword, G.A. and Kang, L., 2012. Mitochondrial genomes reveal the global phylogeography and dispersal routes of the migratory locust. *Mol. Ecol.,* **21:** 4344-4358. https://doi.org/10.1111/j.1365-294X.2012.05684.x

Marvaldi, A.E., Duckett, C.N., Kjer, K.M. and Gillespie, J.J., 2009. Structural alignment of 18s and 28s rdna sequences provides insights into phylogeny of Phytophaga (Coleoptera: Curculionoidea and Chrysomeloidea). *Zool. Scr.,* **38:** 63-77. https://doi.org/10.1111/j.1463-6409.2008.00360.x

McDougall, D.N., 2001. *Anoplophora chinensis*. USDA Forest Service, State and Private Forestry. North American Forest Commission Exotic Forest Pest Information System (NAFC-ExFor).

Meiklejohn, K.A., Danielson, M.J., Faircloth, B.C., Glenn, T.C., Braun, E.L. and Kimball, R.T., 2014. Incongruence among different mitochondrial regions: A case study using complete mitogenomes. *Mol. Phylogenet. Evol.,* **78:** 314-323. https://doi.org/10.1016/j.ympev.2014.06.003

Nie, R.E. and Yang, X.K., 2013. Higher level phylogeny of Coleoptera based on molecular methods: current status and problems. *Acta entomol. Sin.,* **56:** 1055-1062.

ØDegaard, F., 2000. How many species of arthropods? Erwin's estimate revised. *Biol. J. Linn. Soc.,* **71:** 583-597. https://doi.org/10.1111/j.1095-8312.2000.tb01279.x

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

Perna, N.T. amd Kocher, T.D., 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *J. mol. Evol.,* **41:** 353-358. https://doi.org/10.1007/BF01215182

Posada, D. and Buckley, T.R., 2004. Model selection and model averaging in phylogenetics:

Advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. *Syst. Biol.,* **53:** 793-808. https://doi.org/10.1080/10635150490522304

Posada, D., 2008. J model test: Phylogenetic model averaging. *Mol. Biol. Evol.,* **25:** 1253-1256. https://doi.org/10.1093/molbev/msn083

Qian, Y.H., Wu, H.Y., Ji, X.Y., Yu, W.W. and Du, Y.Z., 2014. Mitochondrial genome of the stonefly *Kamimuria wangi* (Plecoptera: Perlidae) and phylogenetic position of Plecoptera based on mitogenomes. *PLoS One,* **9:** e86328. https://doi.org/10.1371/journal.pone.0086328

Rambaut, A. and Drummond, A.J., 2007. Tracer v. 1.5. Available at http://www.beast.bio.ed.ac. uk/Tracer.

Rambaut, A., 2009. FigTree v1.3.1. Available at http://tree.bio.ed.ac.uk/software/ (accessed on 31 September 2017).

Richard, H.E. and Gregory, P., 2002. Longhorned beetles of the genus *Anoplophora* and Lithography: Alien invaders in the eye of the beholder. *Am. Entomol.,* **48:** 200-206. https://doi.org/10.1093/ae/48.4.200

Robertson, J.A., Ślipiński, A., Moulton, M., Shockley, F.W., Giorgi, A., Lord, N.P., Mckenna, D.D., Tomaszewska, W., Forrester, J., Miller, K.B., Whiting, M.F. and Mchugh, J.V., 2015. Phylogeny and classification of Cucujoidea and the recognition of a new superfamily Coccinelloidea (Coleoptera: Cucujiformia). *Syst. Ent.,* **40:** 745-778. https://doi.org/10.1111/syen.12138

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

Sama, G., Buse, J., Orbach, E., Friedman, A., Rittner, O. and Chikatunov, V., 2010. A new catalogue of the Cerambycidae (Coleoptera) of Israel with notes on their distribution and host plants. *Munis Ent. Zool.,* **5:** 1-55.

Schuster, G. and Stern, D., 2009. RNA polyadenylation and decay in mitochondria and chloroplasts. *Prog. mol. Biol. Transl.,* **85:** 393-422. https://doi.org/10.1016/S0079-6603(08)00810-6

Song, H., Sheffield, N.C., Cameron, S.L., Miller, K.B. and Whiting, M.F., 2010. When phylogenetic assumptions are violated: base compositional heterogeneity and among-site rate variation in beetle mitochondrial phylogenomics. *Syst. Ent.,* **35:** 429-448. https://doi.org/10.1111/j.1365-3113.2009.00517.x

Stewart, J.B. and Beckenbach, A.T., 2003. Phylogenetic and genomic analysis of the complete mitochondrial DNA sequence of the spotted asparagus beetle *Crioceris duodecimpunctata*. *Mol. Phylogenet. Evol.,* **26:** 513-526. https://doi.org/10.1016/S1055-7903(02)00421-9

Stewart, J.B. and Beckenbach, A.T., 2005. Insect mitochondrial genomics: The complete mitochondrial genome sequence of the meadow spittlebug *Philaenus spumarius* (Hemiptera: Auchenorrhyncha: Cercopoidae). *Genome,* **48:** 46-54. https://doi.org/10.1139/g04-090

Sun, Y., Chen, C., Gao, J., Abbas, M.N., Kausar, S., Qian, C., Wang, L., Wei, G.Q., Zhu, B.J. and Liu, C.L., 2017. Comparative mitochondrial genome analysis of *Daphnis nerii* and other lepidopteran inects reveals conserved mitochondrial genome organization and phylogenetic relationships. *PLoS One,* **12:** e0178773. https://doi.org/10.1371/journal.pone.0178773

Taanman, J.W., 1999. The human mitochondrial genome: structure, transcription, translation and replication. *BBA-Bioenerg.,* **14:** 103-123. https://doi.org/10.1016/S0005-2728(98)00161-3

Talavera, G. and Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.,* **56:** 564-577. https://doi.org/10.1080/10635150701472164

Timmermans, M.J., Lees, D.C. and Simonsen, T.J., 2014. Towards a mitogenomic phylogeny of Lepidoptera. *Mol. Biol. Evol.,* **7:** 169-178. https://doi.org/10.1016/j.ympev.2014.05.031

Timmermans, M.J.T.N., Barton, C., Haran, J., Ahrens, D., Culverwell, C.L., Ollikainen, A., Dodsworth, S., Foster, P.G., Bocak, L., Vogler, A.P., 2016. Family-level sampling of mitochondrial genomes in coleoptera: Compositional heterogeneity and phylogenetics. *Genome Biol. Evol.,* **8:** 161-175. https://doi.org/10.1093/gbe/evv241

Wang, C., Feng, Y. and Chen, X., 2012. Complete sequence and gene organization of the mitochondrial genome of *Batocera lineolate* Chevrolat (Coleoptera: Cerambycidae). *Chin. Sci. B-Chin.,* **57:** 78-85. https://doi.org/10.1007/s11434-012-5271-3

Wang, C.Y., Feng, Y. and Chen, X.M., 2013. Complete coding region of the mitochondrial genome of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). *Zool. Sci.,* **30:** 570-576. https://doi.org/10.2108/zsj.30.570

Wang, J., Dai, X., Xu, X., Zhang, Z., Yu, D., Storey, K.B. and Zhang, J., 2019. The complete mitochondrial genomes of five longicorn beetles (Coleoptera: Cerambycidae) and phylogenetic relationships within Cerambycidae. *PeerJ,* **7:** e7633. https://doi.org/10.7717/peerj.7633

Wang, J.J., Yang, M.F., Dai, R.H., Li, H. and Wang, X.Y., 2018. Characterization and phylogenetic implications of the complete mitochondrial genome of Idiocerinae (Hemiptera: Cicadellidae). *Int. J. Biol. Macromol.,* **120:** 2366-2372. https://doi.org/10.1016/j.ijbiomac.2018.08.191

Wang, Q. and Tang, G., 2018. The mitochondrial genomes of two walnut pests, *Gastrolina depressa depressa* and *G. depressa thoracica* (Coleoptera: Chrysomelidae) and phylogenetic analyses. *PeerJ,* **6:** e4919. https://doi.org/10.7717/peerj.4919

Wang, Y., Cao, J.J., Li, N., Ma, G.Y. and Li, W.H., 2019. The first mitochondrial genome from Scopuridae (Insecta: Plecoptera) reveals structural features and phylogenetic implications. *Int. J. Biol. Macromol.,* **122:** 893-902. https://doi.org/10.1016/j.ijbiomac.2018.11.019

Wolstenholme, D.R., 1992. Animal mitochondrial DNA: Structure and evolution. *Int. Rev. Cytol.,* **141:** 173-216. https://doi.org/10.1016/S0074-7696(08)62066-5

Wu, H.Y., Ji, X.Y., Yu, W.W. and Du, Y.Z., 2014. Complete mitochondrial genome of the stonefly *Cryptoperla stilifera* Sivec (Plecoptera: Peltoperlidae) and the phylogeny of Polyneopteran insects. *Gene,* **537:** 177-183. https://doi.org/10.1016/j.gene.2013.12.044

Yao, J., Yang, H. and Dai, R., 2017. Characterization of the complete mitochondrial genome of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae) with phylogenetic analysis. *Genetica,* **145:** 397-408. https://doi.org/10.1007/s10709-017-9975-9

Yin, H., Zhi, Y.C., Jiang, H.D., Wang, P.X., Yin, X.C. and Zhang, D.C., 2012. The complete mitochondrial genome of *Gomphocerus tibetarus* Uvarov, (Orthoptera: Acridiaea: Gomphocerinae). *Gene,* **492:** 214-218. https://doi.org/10.1016/j.gene.2011.12.020

Yuan, M., Zhang, Q., Zhang, L., Guo, Z., Liu, Y., Shen, Y. and Shao, R.F., 2016. High-level phylogeny of the coleoptera inferred with mitochondrial genome sequences. *Mol. Phylogenet. Evol.,* **104:** 99-111. https://doi.org/10.1016/j.ympev.2016.08.002

Yukuhiro, K., Sezutsu, H., Itoh, M., Shimizu, K. and Banno, Y., 2002. Significant levels of sequence divergence and gene rearrangements have occurred between the mitochondrial genomes of the wild mulberry silkmoth, *Bombyx mandarina*, and its close relative, the domesticated silkmoth, *Bombyx mor*. *Mol. Biol. Evol.,* **19:** 1385-1389. https://doi.org/10.1093/oxfordjournals.molbev.a004200

Zhang, D.X. and Hewitt, G.M., 1997. Insect mitochondrial control region: A review of its structure, evolution and usefulness in evolutionary studies. *Biochem. Syst. Ecol.,* **25:** 99-120. https://doi.org/10.1016/S0305-1978(96)00042-7

Zhang, S.Q., Che, L.H., Li, Y., Liang, D., Pang, H., Ślipiński, A. and Zhang, P., 2018. Evolutionary history of Coleoptera revealed by extensive sampling of genes and species. *Nat. Commun.,* **9:** 205. https://doi.org/10.1038/s41467-017-02644-4

Zhang, Y., Feng, S., Fekrat, L., Jiang, F., Khathutshelo, M. and Li, Z., 2019. The first two complete mitochondrial genome of *Dacus bivittatus* and *Dacus ciliatus* (Diptera: Tephritidae) by next-generation sequencing and implications for the higher phylogeny of Tephritidae. *Int. Rev. Cytol.,* **140:** 469-476. https://doi.org/10.1016/j.ijbiomac.2019.08.076

Zhang, Z.Q., 2013. Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. *Zootaxa,* **37:** 1-82.

Zhou, X., Han, H., Pang, B. and Zhang, P., 2016. The complete mitochondrial genome of *Galeruca daurica* (Joannis) (Coleoptera: Chrysomelidae). *Mitochondrial DNA A,* **27:** 2891-2892. https://doi.org/10.3109/19401736.2015.1060424

Zhu, J.Y., Yang, P., Zhang, Z., Wu, G.X. and Yang, B., 2013. Transcriptomic immune response of Tenebrio molitor pupae to parasitization by *Scleroderma guani*. *PLoS One,* **8:** 173-185. https://doi.org/10.1371/journal.pone.0054411

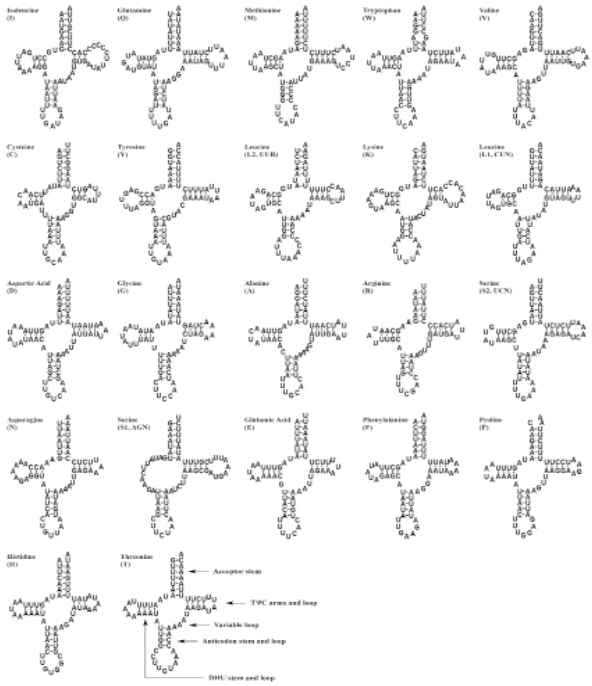
Pakistan J. Zool., pp 1-19, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220824100800

Supplementary Material

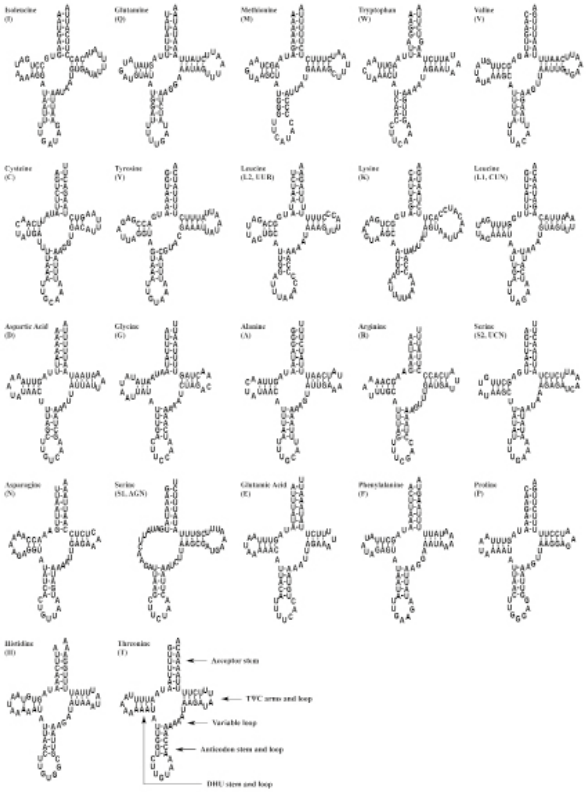
Comparative Mitgenome Analysis of *Anoplophora horsfieldi* and Other Chrysomeloidea, Cucujiformia Insects Reveals Conserved Mitogenome Organization and Phylogeny

Haifen Qin[1,2], Yujia Liu[1], Yujie Zhang[1], Jingfeng Liu[1], Zhenkun Zhao[1,2] and Lichun Jiang[1,2]*

[1]Key Laboratory for Molecular Biology and Biopharmaceutics, School of Life Science and Technology, Mianyang Normal University, Mianyang, Sichuan, 621000, P.R. China.
[2]Ecological Security and Protection Key Laboratory of Sichuan Province, Mianyang Normal University, Mianyang, Sichuan, 621000, P.R. China.



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.

* *Corresponding author: jiang_lichun@126.com*
0030-9923/2022/0001-0001 $ 9.00/0

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	*Anoplophora*	*Anoplophora horsfieldi*	15,796	MN248534
2	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Lamiini	*Anoplophora*	*Anoplophora horsfieldi*	15,837	MW364565
3	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Lamiini	*Anoplophora*	*Anoplophora glabripennis*	15,774	NC_008221
4	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Lamiini	*Anoplophora*	*Anoplophora chinensis*	15,805	NC_029230
5	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Monochamini	*Monochamus*	*Monochamus alternatus*	15,874	NC_024652
6	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Monochamini	*Monochamus*	*Monochamus alternatus*	14,649	JX987292
7	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Batocerini	*Batocera*	*Batocera lineolata*	15,418	JN986793
8	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Batocerini	*Batocera*	*Batocera lineolata*	15,420	MF521888
9	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Agniini	*Psacothea*	*Psacothea hilaris*	15,856	FJ424074
10	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lamiinae	Saperdini	*Thyestilla*	*Thyestilla gebleri*	15,505	NC_034752
11	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Cerambycinae	Cerambycini	*Aeolesthes*	*Aeolesthes oenochrous*	15,747	AB703463
12	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Cerambycinae	Cerambycini	*Massicus.*	*Massicus raddei*	15,858	NC_023937
13	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Cerambycinae	Obriini	*Obrium*	*Obrium* sp. NS-2015	15,680	KT945156
14	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Prioninae	Prionini	*Dorysthenes*	*Dorysthenes paradoxus*	15,922	NC_037927
15	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Prioninae	Callipogonini	*Callipogon*	*Callipogon relictus*	15,742	NC_037698
16	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Lepturinae	Lepturini	*Anastrangalia*	*Anastrangalia sequensi*	16,269	NC_038090
17	Coleoptera	Cucujiformia	Chrysomeloidea	Cerambycidae	Disteniinae		*Disteniazteca*	*Disteniazteca fimbriata*	9,776	JX221000
18	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae	Diabroticina	*Diabrotica*	*Diabrotica barberi*	16,366	NC_022935
19	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae	Diabroticina	*Diabrotica*	*Diabrotica virgifera virgifera*	16,650	KF658070
20	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae	Galerucites	*Galeruca.*	*Galeruca daurica*	16,615	NC_027114
21	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae		*Paleosepharia*	*Paleosepharia posticata*	15,729	NC_033532
22	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Galerucinae	Alticini	*Agasicles*	*Agasicles hygrophila*	15,917	NC_028332
23	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Cassidinae		*Rhadinosa.*	*Rhadinosa nigrocyanea*	17,965	NC_037218
24	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Cassidinae		*Agonita*	*Agonita chinensis*	16,395	MF351622
25	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Chrysomelinae	Chrysomelini	*Gonioctena*	*Gonioctena intermedia*	18,064	MF563963
26	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Chrysomelinae	Chrysomelini	*Gonioctena*	*Gonioctena intermedia*	18,293	MF563962
27	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Chrysomelinae	Chrysomelini	*Gastrolina*	*Gastrolina thoracica*	16,109	NC_036108
28	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Bruchinae	Bruchini	*Acanthoscelides*	*Acanthoscelides obtectus*	16,130	NC_035677
29	Coleoptera	Cucujiformia	Chrysomeloidea	Chrysomelidae	Criocerinae		*Crioceris*	*Crioceris duodecimpunctata*	15,880	AF467886
30	Coleoptera	Cucujiformia	Chrysomeloidea	Vesperidae	Philinae	Philini	*Spiniphilus*	*Spiniphilus spinicornis*	15,707	NC_029515

Table continued on next page.....

No.	Order	Suborder	Superfamily	Family	Subfamily	Tribes	Genus	Species	Species	Accession no.
31	Coleoptera	Cucujiformia	Chrysomeloidea	Orsodacnidae			*Orsodacne*	*Orsodacne lineola*	9,828	JX220994
32	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae		*Sitophilus*	*Sitophilus zeamais*	18,517	KU757289
33	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae		*Sitophilus*	*Sitophilus oryzae*	17,602	KX373615
34	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae		*Sitophilus*	*Sitophilus zeamais*	18,105	KX373614
35	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae		*Rhynchophorus*	*Rhynchophorus ferrugineus*	15,924	KT428893
36	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae		*Sphenophorus*	*Sphenophorus* sp. BYU-CO246	15,052	GU176342
37	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Dryophthorinae		*Cyrtotrachelus*	*Cyrtotrachelus buqueti*	15,035	NC_039705
38	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae		*Eucryptorrhynchus*	*Eucryptorrhynchus brandti*	15,597	KP455482
39	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae		*Eucryptorrhynchus*	*Eucryptorrhynchus scrobiculatus*	15,195	KP455510
40	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae		*Eucryptorrhynchus*	*Eucryptorrhynchus brandti*	15,122	NC_025945
41	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae		*Eucryptorrhynchus*	*Eucryptorrhynchus brandti*	16,919	KR088970
42	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cryptorhynchinae		*Eucryptorrhyn-chus*	*Eucryptorrhynchus scrobiculatus*	15,628	NC_026719
43	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Entiminae	Naupactini	*Naupactus*	*Naupactus xanthographus*	15,026	GU176345
44	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Entiminae	Tanymecini	*Sympiezomias*	*Sympiezomias velatus*	15,592	MF383367
45	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Entiminae	Cyphicerini	*Myllocerinus*	*Myllocerinus aurolineatus*	17,762	NC_040931
46	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Entiminae	Polydrusini	*Pachyrhinus*	*Pachyrhinus yasumatsui*	16,472	MF807224
47	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Curculioninae	Anthonomini	*Anthonomus*	*Anthonomus rubi*	17,476	NC_044714
48	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Curculioninae	Anthonomini	*Anthonomus*	*Anthonomus rectirostris*	17,676	NC_044713
49	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Curculioninae	Anthonomini	*Anthonomus*	*Anthonomus pomorum*	17,093	NC_044712
50	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Curculioninae	Anthonomini	*Anthonomus*	*Anthonomus eugenii*	17,257	NC_044711
51	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Curculioninae	Curculionini	*Curculio*	*Curculio davidi*	16,852	NC_034293
52	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Molytinae	Hylobiini	*Hylobitelus*	*Hylobitelus xiaoi*	16,123	NC_022680
53	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Cyclominae		*Aegorhinus*	*Aegorhinus superciliosus*	15,121	NC_027577
54	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Platypodinae		*Euplatypus*	*Euplatypus parallelus*	16,095	KX711704
55	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Alcidinae		*Alcidodes*	*Alcidodes juglans*	15,638	NC_041669
56	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Ceutorhynchinae		*Ceutorhynchus*	*Ceutorhynchus obstrictus*	20,124	NC_045101
57	Coleoptera	Cucujiformia	Curculionoidea	Curculionidae	Scolytinae		*Scolytinae*	*Scolytinae* sp. BMNH 1274287	17,064	KT696206
58	Coleoptera	Cucujiformia	Curculionoidea	Attelabidae	Rhynchitinae		*Cyllorhynchites*	*Cyllorhynchites ursulus*	14,508	MH156809
59	Coleoptera	Cucujiformia	Curculionoidea	Brentidae	Apioninae		*Lepidapion*	*Lepidapion squamigerum*	18,562	MN459662
60	Coleoptera	Cucujiformia	Tenebrionoidea	Tenebrionidae	Tenebrionidae incertae sedis		*Tribolium*	*Tribolium castaneum*	15,881	AJ312413

Table continued on next page.....

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

Table continued on next page.....

No.	Order	Suborder	Superfamily	Family	Subfamily	Tribes	Genus	Species	Species	Accession no.
90	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	*Cheilomenes*	*Cheilomenes sexmaculata*	17,192	KM244706
91	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	*Propylea*	*Propylea japonica*	15,027	KM244660
92	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Halyziini	*Halyziini*	*Halyziini* sp. HA	14,431	MG584728
93	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	*Coccinella*	*Coccinella transversoguttata*	15,806	MG584726
94	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	*Coelophora*	*Coelophora saucia*	11,942	MN053056
95	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	*Calvia*	*Calvia championorum*	17,575	KX132085
96	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Coccinellinae	Coccinellini	*Propylea*	*Propylea* sp. HSL-2016	15,915	KX132084
97	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Epilachninae	Epilachnini	*Henosepilachna*	*Henosepilachna pusillanima*	16,216	NC_023469
98	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Epilachninae	Epilachnini	*Henosepilachna*	*Henosepilachna vigintioctopunctata*	17,057	NC_041172
99	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Epilachninae	Epilachnini	*Subcoccinella*	*Subcoccinella vigintiquatuorpunctata*	14,645	KT780695
100	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Epilachninae	Epilachnini	*Afissula*	*Afissula* sp. XL-2019	15,339	MN053057
101	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Epilachninae	Epilachnini	*Epilachna*	*Epilachna admirabilis*	17,445	MN053053
102	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae	Scymninae	Scymnini	*Cryptolaemus*	*Cryptolaemus montrouzieri*	10,914	KT878321
103	Coleoptera	Cucujiformia	Coccinelloidea	Coccinellidae			*unclassified Coccinellidae*	Coccinellidae sp. 1 EF-2015	16,631	KT780638
104	Coleoptera	Cucujiformia	Cucujoidea	Nitidulidae	Nitidulinae		*Aethina*	*Aethina tumida*	16,576	NC_036104
105	Coleoptera	Cucujiformia	Cucujoidea	Nitidulidae	Carpophilinae		*Carpophilus*	*Carpophilus dimidiatus*	15,717	MN604384
106	Coleoptera	Cucujiformia	Cucujoidea	Nitidulidae	Carpophilinae		*Carpophilus*	*Carpophilus pilosellus*	15,686	MN604383
107	Coleoptera	Cucujiformia	Cucujoidea	Laemophloeidae			*Cryptolestes*	*Cryptolestes pusillus*	15,502	NC_028204
108	Coleoptera	Cucujiformia	Cucujoidea	Laemophloeidae			*Cryptolestes*	*Cryptolestes ferrugineus*	15,511	NC_028203
109	Coleoptera	Cucujiformia	Cucujoidea	Laemophloeidae			*Cryptolestes*	*Cryptolestes turcicus*	15,517	KT070712
110	Coleoptera	Cucujiformia	Cucujoidea	Phloeostichidae			*Priasilpha*	*Priasilpha obscura*	16,603	EU877952
111	Coleoptera	Cucujiformia	Cucujoidea	Bothrideridae			*Dastarcus*	*Dastarcus helophoroides*	15,878	NC_024271
112	Coleoptera	Cucujiformia	Cucujoidea	Cucujidae			*Cucujus*	*Cucujus clavipes*	15,642	GU176341
113	Coleoptera	Cucujiformia	Cucujoidea	Erotylidae			*unclassified Erotylidae*	Erotylidae sp. BMNH 1274392	16,553	KT696227
114	Coleoptera	Cucujiformia	Cleroidea	Cleridae	Clerinae		*Trichodes*	*Trichodes sinae*	16,047	NC_033340
115	Coleoptera	Cucujiformia	Cleroidea	Chaetosomatidae			*Chaetosoma*	*Chaetosoma scaritides*	15,511	NC_011324
116	Coleoptera	Cucujiformia	Cleroidea	Prionoceridae			*Idgia*	*Idgia oculata*	15,805	NC_044896
117	Coleoptera	Cucujiformia	Lymexyloidea	Lymexilonidae	Melittomminae		*Melittomma*	*Melittomma* sp. MEL01	9,674	JX412776
118	Coleoptera	Scarabaeiformia	Scarabaeoidea	Lucanidae	Lucaninae		*Prosopocoilus*	*Prosopocoilus gracilis*	16,736	KP735805
119	Coleoptera	Scarabaeiformia	Scarabaeoidea	Scarabaeidae	Melolonthinae		*Cheirotonus*	*Cheirotonus jansoni*	17,249	NC_023246
120	Coleoptera	Staphyliniformia	Staphylinoidea	Silphidae	Silphinae		*Necrophila*	*Necrophila americana*	16,902	NC_018352

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

Gene/region	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
1st	36.97	11.26	34.33	17.43	71.30	-0.037	0.215
2nd	47.59	17.54	21.05	13.82	68.63	-0.387	-0.119
3rd	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/region	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
1st	36.01	12.43	33.37	18.19	69.38	-0.038	0.188
2nd	47.36	17.65	20.92	14.07	68.27	-0.387	-0.113
3rd	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/region	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
1st	36.13	12.09	34.13	17.66	70.25	-0.028	0.187
2nd	47.38	17.74	21.12	13.76	68.50	-0.383	-0.126
3rd	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%).