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ORIGINAL ARTICLE

Characterization and Phylogenetic Analysis of the Complete Mitochondrial Genome of *Saturnia japonica*

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Abstract

The complete mitochondrial genome (mitogenome) of *Saturnia japonica* (Lepidoptera: Saturniidae) was sequenced and annotated. It is a circular molecule of 15, 376 bp, composed of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNA), and an adenine (A) + thymine (T)-rich region. All protein-coding genes (PCGs) are initiated by the ATN codon except for cytochrome c oxidase subunit 1 (*cox1*) gene that is seemingly initiated by the CGA codon. Except for *cox2* and *nad4*, which were terminated by incomplete stop codon T or TA, the rest were terminated by canonical stop codon TAA. The A + T-rich region is high conservative, including 'ATAGA' motif followed by a 19 bp poly-T stretch, a microsatellite-like element $(AT)_9$ and also a poly-A element, with a total length of 332 bp. The *Asn* codon was the most frequently used codon, followed by *Ile, Leu2, Lys, Met, Phe,* and *Tyr*, while *Cys* was the least frequently used codon. Phylogenetic relationships analysis based on the 13 PCGs by using maximum likelihood (ML) and neighbor Joining (NJ) revealed that *S. japonica* belongs to the Saturniidae family. In this study, the annotation and characteristics of the mitogenome of *S. japonica* were resolved for the first time, which laid a foundation for species classification and the molecular evolution of Lepidoptera: Saturniidae.

Keywords *Saturnia japonica* · Mitochondrial genome · Genome organization · Phylogeny

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Introduction

Saturnia japonica (Lepidoptera) is one of the most precious wild silkworms, mainly distributed in China, Japan, and Korea, which typically consumes the leaves from walnut or chestnut (Chen et al. 2020). *S. japonica* can spin fluorescent and scintillating silk, which is often used as a raw material for various anti-counterfeiting signs and high-grade clothing (Li et al. 2014). In addition, *S. japonica* is also a kind of resource insect. Its pupa is often used as an edible insect for supplement protein.

Insect mitogenome DNA (mtDNA) is typically composed of 37 conserved genes. It is a closed-circular molecule ranging in size from 14 to 19 kilobases (kb), including the very short or non-existent interval between genes (Jiang et al. 2009). It contains 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and 2 ribosomal RNAs (*rrnL* and *rrnS*) (Boore 1999; Wolstenholme 1992). In addition, there is a poorly conserved adenine (A)+thymine (T)-rich region, which contains the initiation sites of genome transcription and replication (Cameron 2014; Kim et al. 2006; Shadel and Clayton 1993; Lu et al. 2013). This control region is also considered to control transcription initiation and genome replication (Wolstenholme 1992). MtDNA is a powerful tool for species identification (Dowton et al. 2002), genomic evolution (Miya et al. 2001), phylogeography (Avise et al. 1987), and molecular evolution (Forstén 1991) based on its simple genomic organization, high rate of evolutionary, and almost clear homology (Zhang et al. 2015).

Lepidoptera is a gigantic family with more than 160000 species, which can be divided into 45–48 superfamilies (Hao et al. 2012). Saturniidae contains 800 species distributed worldwide and more than 44 species recorded in China. In addition to being an important indicator of plant pest control in agriculture and forestry, they are also valuable in the process of plant pollination (Chen et al. 2020). However, only a few species mitogenomes have been completely sequenced, which are publically available in GenBank (Table 1) (Kawaguchi and Nishida 2001, Sun et al. 2017).

It is important to understand the genetic characteristics and phylogenetic status of *S. japonica* for improving its comprehensive utilization. The complete mitogenome of *S. japonica* was sequenced, annotated, and compared with the other Lepidoptera species to elucidate molecular evolution, comparative and evolutionary genomics, phylogenetics, and population genetics (Jiang et al. 2009).

Materials and Methods

Experimental Insects and DNA Extraction

Saturnia japonica pupae were collected from the chestnut trees on the Scenic of Dabie Mountains, Anhui Province, China. Fresh specimens were preserved in 100% ethanol and stored at -80℃. The total DNA was extracted using the

Table 1 Details of the Lepidopteran mitogenomes used in this study

Superfamily	Family	Species	Size (kb)	GenBank accession no
Bombycoidea	Bombycidae	*Bombyx mori*	15.643	NC_002355
	Saturniidae	*Antheraea assama*	15.312	KU301792
		Eriogyna pyretorum	15.327	FJ685653
		Saturnia japonica	15.376	MT614593
		Saturnia jonasii	15.261	MF346379
		Saturnia boisduvalii	15.360	EF622227
		Samia cynthia ricini	15.384	JN215366
	Sphingidae	*Parum colligata*	15.288	NC_039166
Noctuoidea	Lymantriidae	*Lymantria dispar*	15.569	NC_012893
	Noctuidae	*Ctenoplusia agnata*	15.261	KC414791
		Agrotis ipsilon	15.377	KF163965
		Helicoverpa armigera	15.347	GU188273
	Notodontidae	*Phalera flavescens*	15.659	JF440342
	Erebidae	*Hyphantria cunea*	15.481	GU592049
Geometroidea	Geometridae	*Apocheima cinerarium*	15.722	KF836545
	Phthonandria	*Phthonandria atrilineata*	15.499	NC_010522
Pyraloidea	Crambidae	*Chilo suppressalis*	15.395	NC_015612
		Elophila interruptalis	15.351	NC_021756
		Tyspanodes hypsalis	15.329	NC_025569
		Evergestis junctalis	15.438	KP347976
		Glyphodes quadrimaculalis	15.255	KF234079
	Pyralidae	*Lista haraldusalis*	15.213	KF709449
		Hypsopygia regina	15..212	KP327714
Gelechioidea	Gelechiidae	*Tecia solanivora*	15.251	KT326187
	Oecophoridae	*Promalactis suzukiella*	15.507	KM875542
Tortricoidea	Tortricidae	*Grapholita molesta*	15.717	HQ392511
		Lobesia botrana	15.229	KP677508
		Cydia pomonella	15.253	JX407107
Papilionoidea	Papilionidae	*Bhutanitis mansfieldi*	14.994	NC_037863
		Graphium timur	15.226	KJ472924
		Lamproptera meges	15.113	NC_037867
		Graphium chironides	15.235	KP159289
	Nymphalidae	*Timelaea maculata*	15.178	KC572131
		Junonia vestina	15.224	KX267577
	Pieridae	*Catopsilia pomona*	15.142	JX274649
Yponomeutoidea	Plutellidae	*Plutella xylostella*	16.179	JF911819
	Lyonetiidae	*Leucoptera malifoliella*	15.646	NC_018547

Genomic DNA Extraction Kit according to the manufacturer's instructions (Sangon Biotech Co. Ltd, Shanghai, China). The extracted DNA quality was examined by 1% agarose gel electrophoresis (w/v) and then used for the PCR amplification.

Primer Design, PCR Amplification, and Sequencing

Fourteen overlapping fragments pairs of primers were designed based on the conserved nucleotide sequences of known the reported mitogenome of Lepidopteran species and synthesized by General Biosystems Co. Ltd. (Chuzhou, China). The complete list of successful primers is given in Table 2. All PCRs were performed in 50 µL reaction volume, which including 35 µL sterilized distilled water, 5 µL 10 × Taq buffer (Mg^{2+} plus), 4 µL dNTP (2.5 mM), 1.5 µL DNA as template, 2 µL of each primer (10 µM), and 0.5 µL (1 unit) Taq DNA polymerase (TaKaRa Biotechnology Co., Dalian, China). The PCR amplification conditions were as follows: 4 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 40 s at 46–56 °C, and 2–3

Table 2 Details of the primers used to amplify the mitogenome of *S. japonica*

Primer pair	Primer sequences (5′–3′)	Annealing temperature
F1	TAAAAATAAGCTAAATTTAAGCTT	52 °C
R1	TATTAAAATTGCAAATTTTAAGGA	
F2	AGGAGGTCTCCCCCCTTTCTTAGG	48 °C
R2	CATCCTGTACCTGCTCCATTTTCTAC	
F3	AAACTAATAATCTTCAAAATTAT	51 °C
R3	AAAATAATTTGTTCTATTAAAG	
F4	CGTCGTTATTCAGACTATCCAG	51 °C
R4	GACCTGCGATTATATTAGCAG	
F5	CATCTCCATTTGAATGCGG	50 °C
R5	GGGGGATTTATAGGGTATT	
F6	TAAGCTGCTAACTTAATTTTTAGT	53 °C
R6	CCTGTTTCAGCTTTAGTTCATTC	
F7	CCTAATTGTCTTAAAGTAGATAA	4 °C
R7	TGCTTATTCTTCTGTAGCTCATAT	
F8	CTTCGTCTATGTAAACGTTC	50 °C
R8	GTTCATTTGAGGCAATTCTT	
F9	ACTTTAAAAACTTCAAAGAAAAA	48 °C
R9	TCATAATAAATTCCTCGTCCAATAT	
F10	GTAAATTATGGTTGATTAATTCG	47 °C
R10	TGATCTTCAAATTCTAATTATGC	
F11	CCGAAACTAACTCTCTCTCACCT	54 °C
R11	CTTACATGATCTGAGTTCAAACCG	
F12	CAATCCTTTCGTACTAAAAT	50 °C
R12	GTCGTAACAAAGTAGAGG	
F13	TCTAGAAACACTTTCCAGTACCTC	51 °C
R13	AATTTTAAATTATTAGGTGAAATT	
F14	TAATAGGGTATCTAATCCTAGTT	51 °C
R14	ACTTAATTTATCCTATCAGAATAA	

min (depending on putative length of the fragments) at 72 °C, and then a final extension step of 72 °C for 10 min. The PCR products were detected by 1% agarose gel electrophoresis (w/v) and then purified using a DNA gel extraction kit (Sangon Biotech Co. Ltd., Shanghai, China). The purified fragments were ligated into the T-vector (TaKaRa Biotechnology Co., Dalian, China) and transformed into *Escherichia coli* TOP10. The insert DNA-positive recombinant colonies were sequenced bidirectionally at least three times by General Biosystems Co. Ltd. (Chuzhou, China).

Sequence Assembly and Gene Annotation

Sequence annotation was performed by comparing with other Lepidoptera species sequenced previously using blast tools available from the NCBI (https://blast.ncbi.nlm.nih. gov/Blast.cgi) and SeqMan II program from the Lasergene software package (DNASTAR Inc., Madison, USA) (Guo et al. 2019). The protein-coding sequences were translated into putative proteins on the basis of the Invertebrate Mitochondrial Genetic Code. The skewness was measured by the method given by Junqueira et al and describe the base composition of the nucleotide sequence as follows: AT skew = [A–T]/[A+T], GC skew = [G–C]/[G+C] (Junqueira et al. 2004). The relative synonymous codon usage (RSCU) values were calculated by using MEGA7.0 (Tamura et al. 2011).

The tRNA genes were verified using either program tRNA scan-SE Search with the default settings (http://lowelab.ucsc.edu/tRNAscan-SE/) (Tamura et al. 2011), or by manually identifying sequences with the appropriate anticodon capable of folding into the typical cloverleaf secondary structure (Lowe and Eddy 1997). The tandem repeats in the A+T-rich region were determined by the tandem repeats finder program (http://tandem.bu.edu/trf/trf.html) (Dai et al. 2015).

Phylogenetic Analysis

To illustrate the phylogenetic relationship among Lepidoptera insects, 38 complete mitogenomes were downloaded from the GenBank DataBase (Table 1). The mitogenomes of *Cucujus clavipes* (GU176341.1) (Song et al. 2010) and *Cucujus haematodes* (KX087268.1) were downloaded as outgroups. Multiple comparison of the 13 PCGs concatenated nucleotide sequences were conducted using MEGA version 7.0. Then the 13 protein nucleotide sequences were serialized into a group for phylogenetic analysis, which were performed using Maximum Likelihood (ML) and Neighbor Joining (NJ) method based on the Kimura 2-parameter (K2P) model (Kimura et al. 1980). Evolutionary analysis was conducted in the MEGA version7.0 program (Tamura et al. 2011). The ML analysis was used to infer phylogenetic trees with 1000 bootstrap replicates, and Neighbor Joining (NJ) distance analysis was performed using PAUP4b 10 (Thompson et al. 1997). The NJ analysis was done with 1000 bootstrap replicates. The consensus tree was visualized using FigTree v1.4.0 (http://tree.bio.ed.ac.uk/Sofw-are/fgtree/).

Results and Discussions

Genome Structure, Organization and Composition

The mitogenome of *S. japonica* is a circular molecule of 15, 376 bp in length (Fig. 1), which is within the range observed in the entire sequence of Lepidopteran species with the size ranging from 16, 179 bp in *Plutella xylostella* (Plutellidae) to 15, 113 bp in *Lamproptera meges* (Papilionidae) (Table 1) (Swofford 2003). The sequence had been annotated and deposited into GenBank under the accession number MT614593. The mitogenome structure of *S. japonica* conforms to the classic 38 regions of the Lepidopteran mitogenome, including 13 protein-encoding regions, 22 tRNA-encoding regions, two rRNA-encoding regions, and a large non-coding-region with high A + T-rich composition (Table 3) (Wei et al. 2013; Rand 1993). The arrangement and orientation of genes in the mitogenome

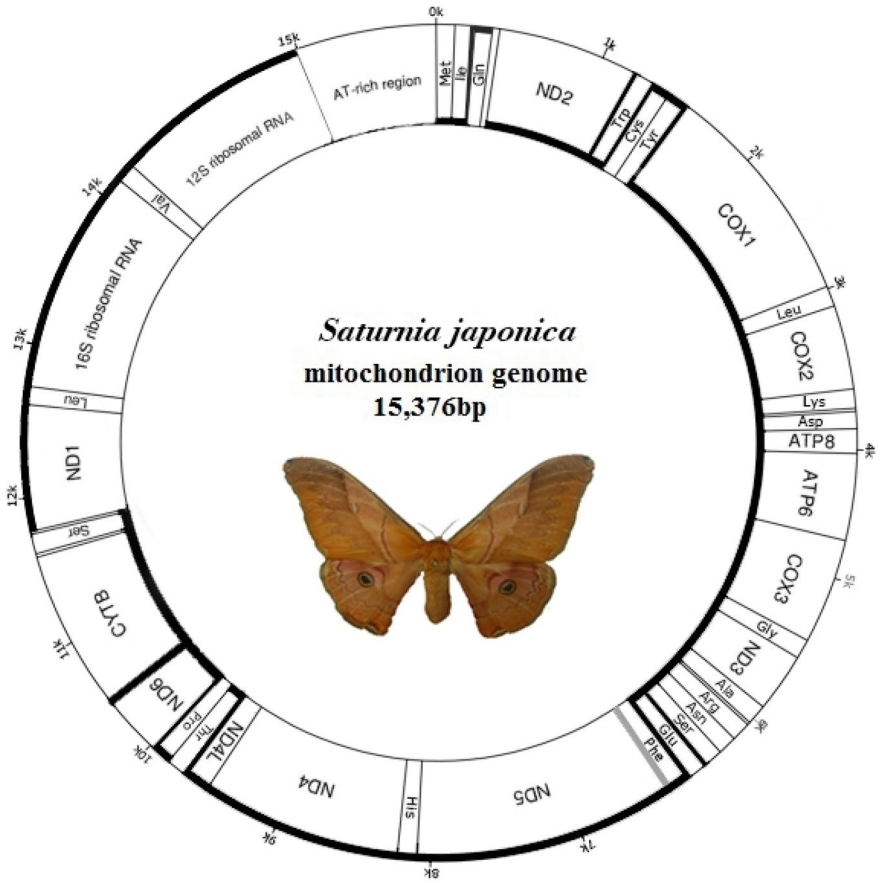


Fig. 1 Map of the mitogenome of *S. japonica*

Table 3 List of the annotated mitochondrial genes of *S. japonica*

Gene	Direct on	Location	Size	Anti-codon	Start codon	Stop codon	Intergenic nucleotides
tRNA^{Met}	F	1–67	67	CAT			1
tRNA^{Ile}	F	69–132	64	GAT			− 3
tRNA^{Gln}	R	130–198	69	TTG			53
nad2	F	252–1265	1014		ATT	TAA	8
tRNA^{Trp}	F	1274–1342	69	TCA			− 8
tRNA^{Cys}	R	1335–1397	63	GCA			0
tRNA^{Tyr}	R	1398–1462	65	GTA			11
cox1	F	1474–3012	1539		CGA	TAA	− 6
tRNA$^{Leu(UUR)}$	F	3007–3075	69	TAA			0
cox2	F	3076–3757	682		ATG	T	0
tRNA^{Lys}	F	3758–3828	71	CTT			22
tRNA^{Asp}	F	3851–3923	73	GTC			0
atp8	F	3924–4088	165		ATC	TAA	− 7
atp6	F	4082–4758	677		ATG	TAA	0
cox3	F	4759–5547	789		ATG	TAA	2
tRNA^{Gly}	F	5550–5616	67	TCC			0
nad3	F	5617–5968	352		ATT	TAA	0
tRNA^{Ala}	F	5969–6035	67	TGC			12
tRNA^{Arg}	F	6048–6112	65	TCG			0
tRNA^{Asn}	F	6113–6176	64	GTT			− 1
tRNA$^{Ser(AGN)}$	F	6176–6241	66	GCT			6
tRNA^{Glu}	F	6248–6317	70	TTC			2
tRNA^{Phe}	R	6320–6387	68	GAA			− 17
nad5	R	6371–8128	1758		ATA	TAA	0
tRNA^{His}	R	8129–8194	66	GTG			1
nad4	R	8196–9536	1341		ATC	TA	0
nad4L	R	9537–9826	290		ATA	TAA	6
tRNA^{Thr}	F	9833–9897	65	TGT			0
tRNA^{Pro}	R	9898–9965	68	TGG			2
nad6	F	9968–10,495	528		ATA	TAA	4
cytb	F	10,500–11,660	1161		ATG	TAA	41
tRNA$^{Ser(UCN)}$	F	11,702–11,768	67	TGA			22
nad1	R	11,791–12,729	939		ATA	TAA	1
tRNA$^{Leu(CUN)}$	R	12,731–12,798	68	TAG			0
rrnL	R	12,799–14,205	1407				0
tRNA^{Val}	R	14,206–14,272	67	TAC			0
rrnS	R	14,273–15,044	772				0
A + T-rich Region		15,045–15,376	332				

of *S. japonica* is trnM-trnI-trnQ, which is different from the ancestral gene order trnI-trnQ-trnM (Boore 1999). Most of the 23 genes were transcribed on the majority-coding strand (H-strand) and a few on the minority-coding strand (L-strand). The comparison of *S. japonica* mitogenome composition and skewness level with other sequenced Lepidoptera species is represented in Table 4. The genome composition of *S. japonica* is A: 39.37%, T: 41.30%, G: 7.56%, and C: 11.77%, with a total A + T content of 80.66%. This is within the scope of similar species (A + T bias of 78.26% in *Tecia solanivora* and 82. 56% in *Leucoptera malifoliella*) (Table 4) (Mcknight and Shaffer 1997; Ramirez-Rios et al. 2016). Additionally, it exhibits negative AT skewness (− 0.024) and negative GC skewness (− 0.218). The AT skewness in other Lepidopteran mitogenomes sequenced to date ranges from 0.057 (*A. cinerarium*) to − 0.036 (*T. maculata*) (Wu et al. 2012), while the GC skewness from − 0.247 (*L. dispar*) to − 0.177 (*A. ipsilon*) (Table 4) (Liu et al. 2014). Similarly, the 13 PCGs, tRNA, rRNA, and A + T-rich region of *S. japonica* are all within the range of the observed Lepidoptera.

Table 4 Composition and skewness in different Lepidopteran mitogenomes

Species	Size(kb)	A%	G%	T%	C%	A + T%	AT skewness	GC skewness
Whole genome								
S. japonica	15.376	39.37	7.56	41.3	11.77	80.66	− 0.024	− 0.218
A. assama	15.312	39.35	7.71	40.82	12.11	80.18	− 0.018	− 0.222
P. colligata	15.288	40.74	7.65	40.31	11.29	81.06	0.005	− 0.192
L. dispar	15.569	40.58	7.57	39.3	12.55	79.88	0.016	− 0.247
A. ipsilon	15.377	40.38	7.71	40.87	11.04	81.25	− 0.006	− 0.177
P. flavescens	15.659	40.07	7.87	40.8	11.26	80.87	− 0.009	− 0.177
H. cunea	15.481	40.58	7.55	39.81	12.06	80.39	0.01	− 0.23
A. cinerarium	15.722	41.52	7.79	39.33	11.37	80.85	0.027	− 0.187
P. atrilineata	15.499	40.78	7.67	40.24	11.31	81.02	0.007	− 0.192
C. suppressalis	15.395	40.64	7.39	40.03	11.94	80.67	0.008	− 0.235
E. junctalis	15.438	39.93	7.87	41.15	11.05	81.08	− 0.015	− 0.168
T. solanivora	15.251	38.62	8.44	39.64	13.3	78.26	− 0.013	− 0.224
P. suzukiella	15.507	39.71	7.56	41.77	10.95	81.49	− 0.025	− 0.183
P. nomion	15.362	40.02	7.56	41.3	11.11	81.32	− 0.016	− 0.19
L. botrana	15.229	40.08	7.89	40	12.04	80.08	0.001	− 0.208
G. timur	15.226	39.84	7.89	40.52	11.75	80.36	− 0.008	− 0.197
T. maculata	15.178	39.06	7.63	41.97	11.34	81.03	− 0.036	− 0.196
C. pomona	15.142	39.45	7.64	41.84	11.07	81.29	− 0.029	− 0.183
C. benjaminii	15.272	40.08	7.52	40.7	11.7	80.78	− 0.008	− 0.218
T. renzhiensis	16.173	41.09	7.54	40.2	11.17	81.28	0.011	− 0.194
L. malifoliella	15.646	41.93	6.99	40.63	10.45	82.56	0.016	− 0.199

Protein-Coding Genes and Codon Usage

The 13 protein-coding genes of *S. japonica* mitogenome are 11, 235 bp long (Appendix, Table 5) and account for 73.07% of the total nucleotides. The AT negative skewness (− 0.019) indicates the occurrence of less As than Ts. Nine of these PCGs (*nad2, cox1, cox2, atp8, atp6, cox3, nad3, nad6, and cob*) are coded by the H-strand, while the remaining four PCGs (*nad5, nad4, nad4L, and nad1*) are coded by the L-strand. In addition, twelve of these PCGs begin with ATN (four with ATA, two with ATT, four with ATG, and two with ATC) codons, while the remaining *cox1* gene of *S. japonica* starts with CGA codon as previously documented in *Leucoma salicis* (Wu et al. 2015). Except that *cox2* terminates with a single T and *nad4* terminates with TA, the remaining 11 PCGs use typical TAA termination codon (Table 3). This phenomenon has been reported in most of the sequenced Lepidopteran mitogenomes (Sun et al. 2016). Single T-stop codon generates functional stop codons by polyadenylation of adjacent PCGs and endonuclease recognition of polycistronic pre-mRNA transcription (Liu et al. 2013).

The mitochondrial genome codon usage of ten Lepidopteran insects was analyzed and divided into eight superfamilies: three species belonging to Bombycoidea, and seven belonging to Pyraloidea, Noctuoidea, Papilionoidea, Geometroidea, Yponomeutoidea, Gelechioidea, and Hepialoidea (Fig. 2). The results revealed that *Asn*, *Ile*, *Leu2*, *Lys*, *Met*, *Phe*, and *Tyr* were the most frequently utilized amino acids; however, the *Arg* codon family was the rarest. Codon distributions of three species in *Bombycoidea* are in consistency, and each amino acid has equal contents in different species (Appendix, Fig. 7).

The 13 PCGs of the *S. japonica* mitogenome contain all codons (Appendix, Fig. 8). This is similar to *A. cinerarium* (Wu et al. 2012), *L. dispar*, *T. renzhiensis* (Wich et al. 1986), *T. solanivora* (Mcknight and Shaffer 1997), and *G. timur* (Cao et al. 2012), but in some Lepidopteran insects, high GC content codons are

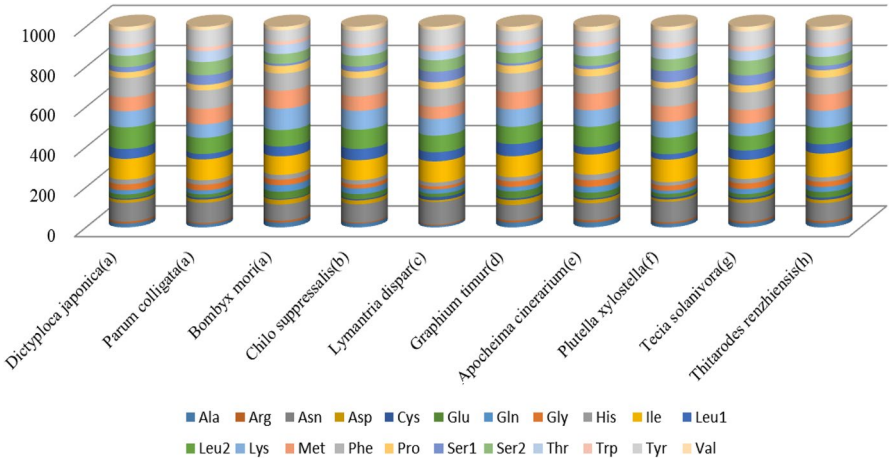


Fig. 2 Comparison of codon usage in the mitogenome of Lepidoptera

abandoned as one of the mitochondrial features (Lu et al. 2013; Chen et al.2016), such as *B. mori* (lack of GCG), *C. suppressalis* (lack of GCG & CGT), *P. colligata* (lack of GCG, GCG & CGT), and *P. xylostella* (lack of GCG) (Swofford 2003).

Ribosomal and Transfer RNA Genes

The mitogenome of *S. japonica* including two typical rRNA genes of Lepidoptera has been sequenced. The large ribosomal gene (*rrnL*) is 1407 bp in length and resided between $tRNA^{Leu}$ (CUN) and $tRNA^{Val}$, whereas the small ribosomal gene (*rrnS*) is only 772 bp, and located between $tRNA^{Val}$ and A + T-rich region (Table 3). The A + T content of the two rRNAs is 84.72%, which ranged from 83.60% (*T. solanivora*) to 86.09% (*L. malifoliella*) of Lepidopterans. In addition, AT skewness (−0.037) and GC skewness (-0.351) of the two rRNAs are negative (Appendix, Table 5), which are located within the range of reported Lepidopteran insects. This was similar to *A. assama*, *A. ipsilon*, and *P. flavescens* and so on.

The *S. japonica* mitochondrial genome harbors an entire set of 22 tRNA genes ranging from 63 bp ($tRNA^{Cys}$) to 73 bp ($tRNA^{Asp}$). 14 of the 22 tRNA genes were coded by the H-strand and remainder eight were coded by the L-strand. The A + T content of the 22 tRNA genes is 81.79%, and AT skewness (− 0.013) and GC skewness (− 0.138) of the two rRNAs are negative (Appendix, Table 5). All tRNAs exhibit typical secondary structure of clover, except for trnS1 lacking the DHU stem (Fig. 3),which is similar to several other previously sequenced Lepidopterans insects (Dai et al. 2016; Liao et al. 2010).

A total of 12 mismatched bps in the *S. japonica* tRNAs were identified, 7 of the 12 G-U wobble pairs scatter throughout the seven tRNAs (two in acceptor stem, four in DHU, and one in anticodon stem). A–A mismatches in the DHU of the tRNG, three U–U mismatch in acceptor stem of the *trnA*, *trnL2*, *trnN*, and one U–U mismatches in anticodon stem of the *trnV* (Fig. 3).

Overlapping and Intergenic Spacer Regions

The mitogenome of *S. japonica* cins 42 bp overlapping nucleotides, which are located in 12 pairs of adjacent genes with the length from 1 to 17 bp and the longest overlap (17 bp) existed between *trnF* and *nad5*. In addition, there is 194 bp intergenic nucleotides (IGN) that are distributed among 26 pairs of adjacent genes ranging from 1 to 53 bp, and the longest spacer sequence was located between *trnQ* and *nad2*, which is usually found in Lepidopteran mitogenomes (Wu et al. 2010). Surprisingly, the seven nucleotides sequence "ATGATAA" is included in the mitochondrial sequence of the ten Lepidopteran insects observed (Fig. 4), common feature across Lepidopteran mitogenomes (Zhu et al. 2013). The 22 bp spacer between *trnS2* (UCN) and *nad1* contains the motif 'ATACTAA,' which is highly conserved region and found in most insect mtDNAs, but Hepialoidea only contains the motif 'ATACTA' (Fig. 5A); hence, the fragment 'ATACTA' is highly conserved region, which may be the peptide-binding site of mitochondrial transcription termination (mtTERM protein) (Taanman 1999).

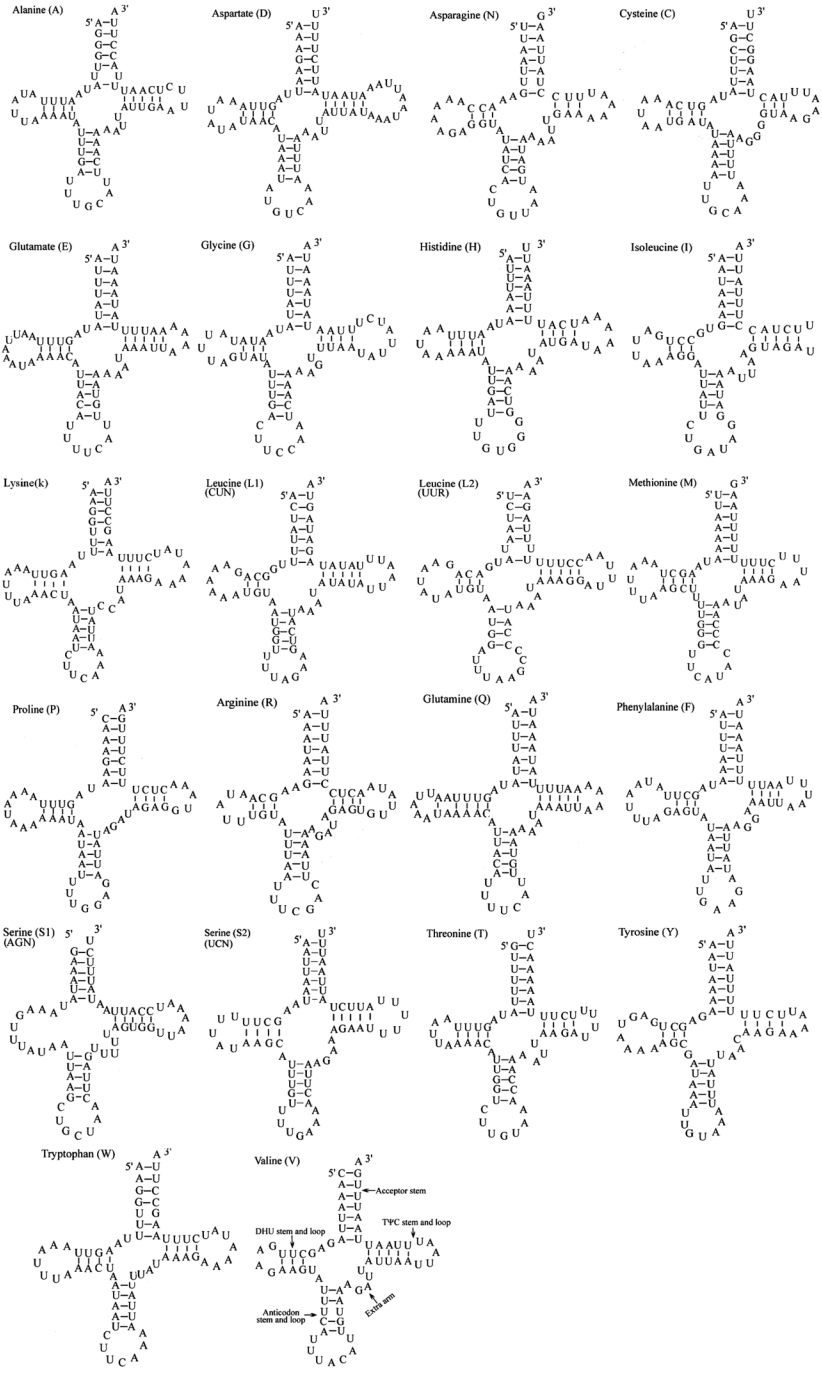


Fig. 3 Putative secondary structures of the 22 tRNAs of the *S. japonica* mitogenome

Fig. 4 Alignment of overlapping region between *atp8* and *atp6* across Lepidoptera and other insects

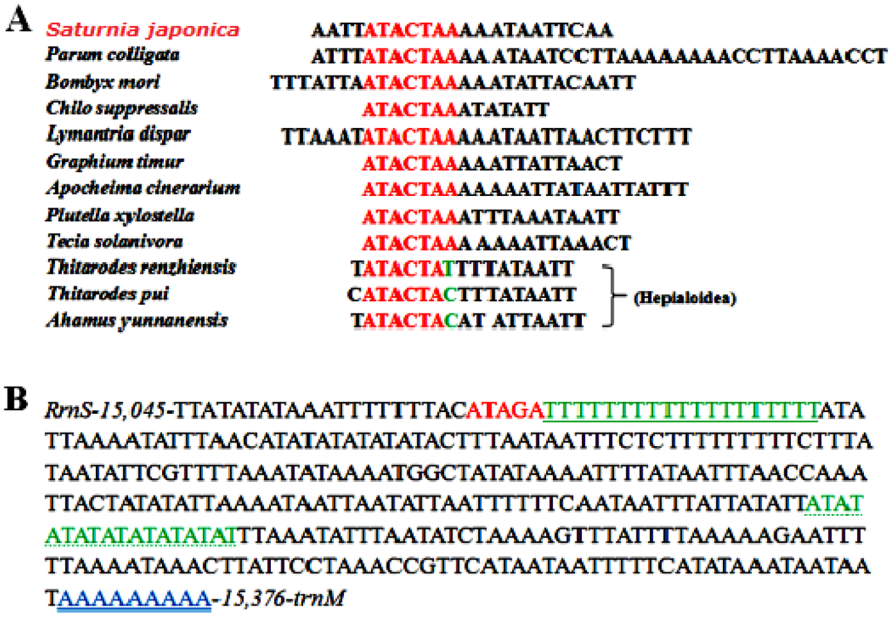


Fig. 5 (**A**) Alignment of the intergenic spacer region between *trnS2* (UCN) and *nad1* of several Lepidopteran insects. The shaded 'ATACTAA' motif is conserved across the Lepidoptera order. (**B**) Features present in the A + T-rich region of *S. japonica***.** The sequence is transcribed in the reverse strand. The ATATG motif is shaded. The poly-T stretch is underlined while the poly-A stretch is boldly underlined. The single-microsatellite T/A repeats sequence is indicated by dotted underlining

The A + T-Rich Region

The A + T-rich region of *S. japonica* mitogenome is located between the *rrnS* and *trnM*, with a length of 332 bp, remarkably shorter than that of *T. renzhiensi* (1367 bp) and longer than *L. botrana* (286 bp) (Appendix, Table 5). This region contains the highest A + T content (91.87%) in the mtDNA, and its AT skewness (− 0.082) and GC skewness (-0.481) are both negative (Appendix, Table 5). Several short repeating sequences scattered throughout the entire region, including the motif 'ATAGA' followed by a 17 bp poly-T stretch, a microsatellite-like $(AT)_9$ element and a poly-A element upstream of *trnM* gene similar to other Lepidopteran mitogenomes (Fig. 5B). The length of poly-T stretch is variable (Lu et al. 2013), while 'ATAGA' region is highly conserved among Lepidoptera species (Cameron and Whiting 2008).

Phylogenetic Analysis

In this study, the nucleotide sequences of the 13 PCGs were concatenated and aligned to reconstruct the phylogenetic relationships among 39 Lepidoptera insects by using Maximum Likelihood (ML), Neighbor Joining (NJ) methods (Chai et al. 2012; Hassanin 2006). Species are clustered by family (Fig. 6). The phylogenetic analysis showed that the *S. japonica* was within the Saturniidae family (Bombycoidea), and it has a closer relationship to *S. boisduvalii* and *S. jonasii.* This is consistent with the conclusion of Kim et al. (2015) and clustered with other superfamilies, ordinal the Geometroidea, Noctuoidea, Pyraloidea, Gelechioidea, Papilionoidea, Tortricoidea, and Yponomeutoidea. The phylogenetic tree constructed by ML and NJ methods showed that *Saturnia japonica, Saturnia boisduvalii, Saturnia jonasii, Eriogyna pyretorum, Antheraea assama*, and *Samia cynthia ricini* belonged to Saturniidae, which was consistent with traditional taxonomic results. Similar phenomena also occur in other superfamilies, and the results of phylogenetic tree analysis are completely consistent with the results of traditional entomological taxonomy.

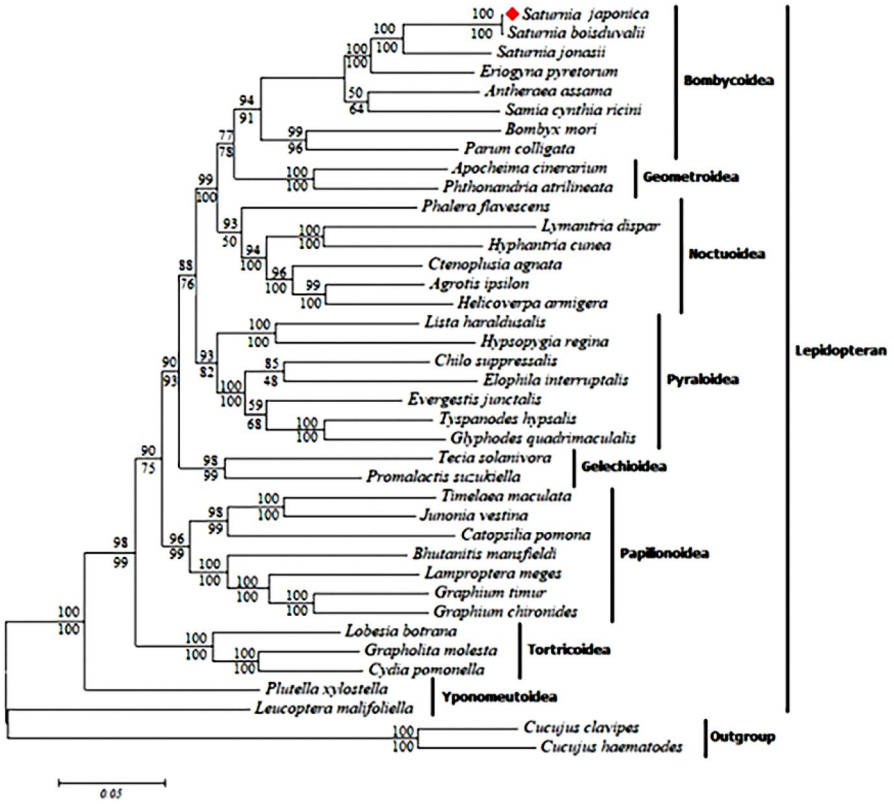
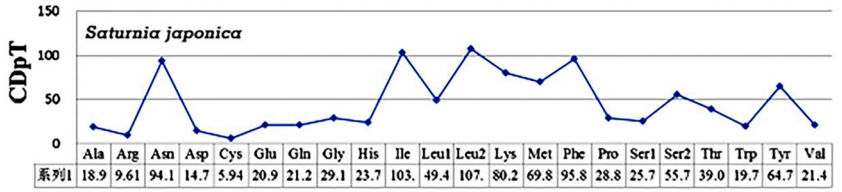


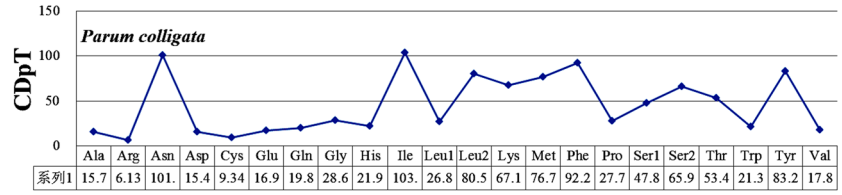
Fig. 6 Phylogenetic relationships among species. Tree constructed using Maximum Likelihood (ML) and Neighbor Joining (NJ) with 1000 bootstrap replicates showed the phylogenetic relationships among 39 species, *Cucujus clavipes* (GU176341.1) and *Cucujus haematodes* (KX087268.1) were used as outgroups. This tree (ML tree), with bootstraps values from ML and NJ is at the nodes above and below, respectively

Appendix

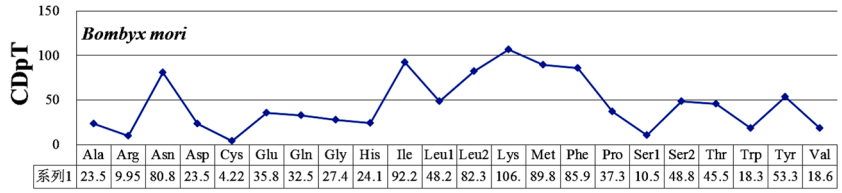
See appendix Figs. 7 and 8; Table 5.



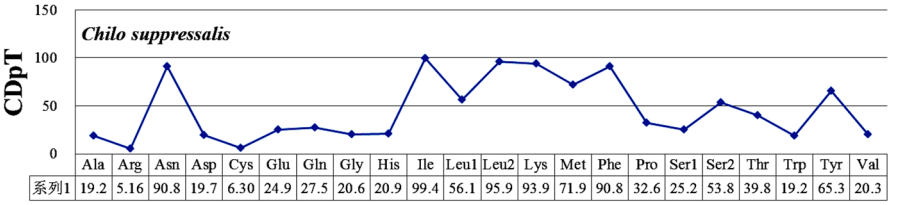
	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	18.9	9.61	94.1	14.7	5.94	20.9	21.2	29.1	23.7	103.	49.4	107.	80.2	69.8	95.8	28.8	25.7	55.7	39.0	19.7	64.7	21.4



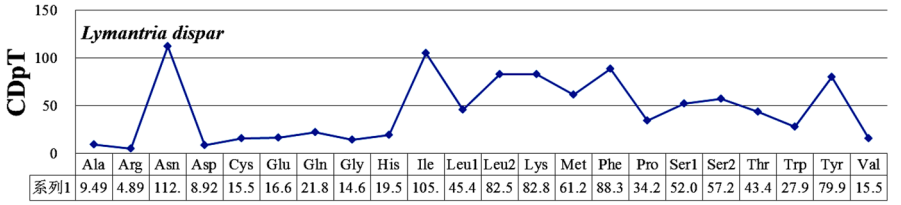
	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	15.7	6.13	101.	15.4	9.34	16.9	19.8	28.6	21.9	103.	26.8	80.5	67.1	76.7	92.2	27.7	47.8	65.9	53.4	21.3	83.2	17.8



	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	23.5	9.95	80.8	23.5	4.22	35.8	32.5	27.4	24.1	92.2	48.2	82.3	106.	89.8	85.9	37.3	10.5	48.8	45.5	18.3	53.3	18.6

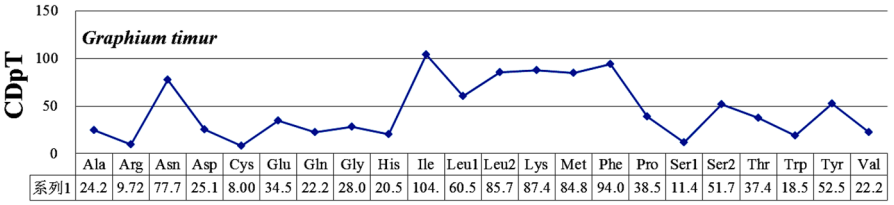


	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	19.2	5.16	90.8	19.7	6.30	24.9	27.5	20.6	20.9	99.4	56.1	95.9	93.9	71.9	90.8	32.6	25.2	53.8	39.8	19.2	65.3	20.3

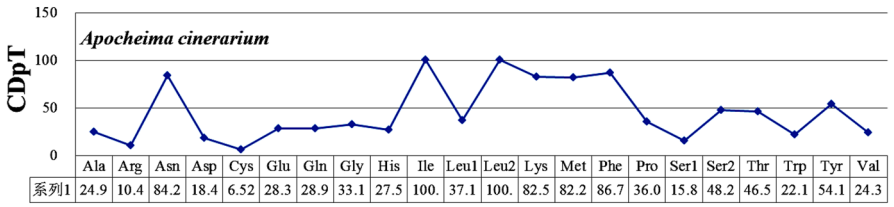


	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	9.49	4.89	112.	8.92	15.5	16.6	21.8	14.6	19.5	105.	45.4	82.5	82.8	61.2	88.3	34.2	52.0	57.2	43.4	27.9	79.9	15.5

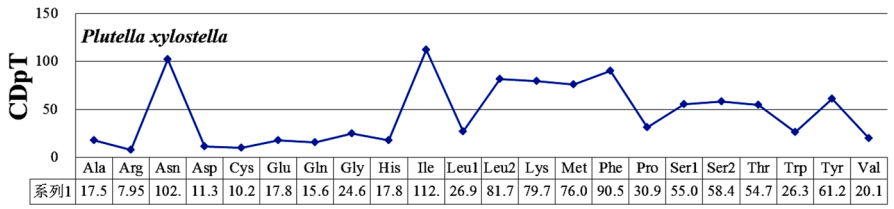
Fig. 7 Codon distribution in the mitogenome of Lepidoptera. *CDspT* codons per thousand codons



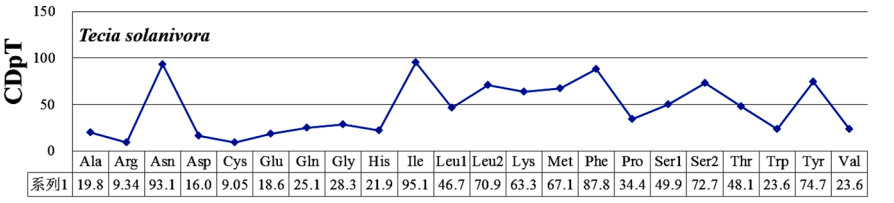
	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	24.2	9.72	77.7	25.1	8.00	34.5	22.2	28.0	20.5	104.	60.5	85.7	87.4	84.8	94.0	38.5	11.4	51.7	37.4	18.5	52.5	22.2



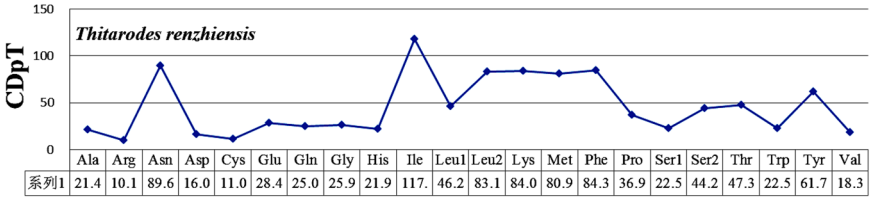
	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	24.9	10.4	84.2	18.4	6.52	28.3	28.9	33.1	27.5	100.	37.1	100.	82.5	82.2	86.7	36.0	15.8	48.2	46.5	22.1	54.1	24.3



	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	17.5	7.95	102.	11.3	10.2	17.8	15.6	24.6	17.8	112.	26.9	81.7	79.7	76.0	90.5	30.9	55.0	58.4	54.7	26.3	61.2	20.1



	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	19.8	9.34	93.1	16.0	9.05	18.6	25.1	28.3	21.9	95.1	46.7	70.9	63.3	67.1	87.8	34.4	49.9	72.7	48.1	23.6	74.7	23.6



	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu1	Leu2	Lys	Met	Phe	Pro	Ser1	Ser2	Thr	Trp	Tyr	Val
系列1	21.4	10.1	89.6	16.0	11.0	28.4	25.0	25.9	21.9	117.	46.2	83.1	84.0	80.9	84.3	36.9	22.5	44.2	47.3	22.5	61.7	18.3

Fig. 7 (continued)

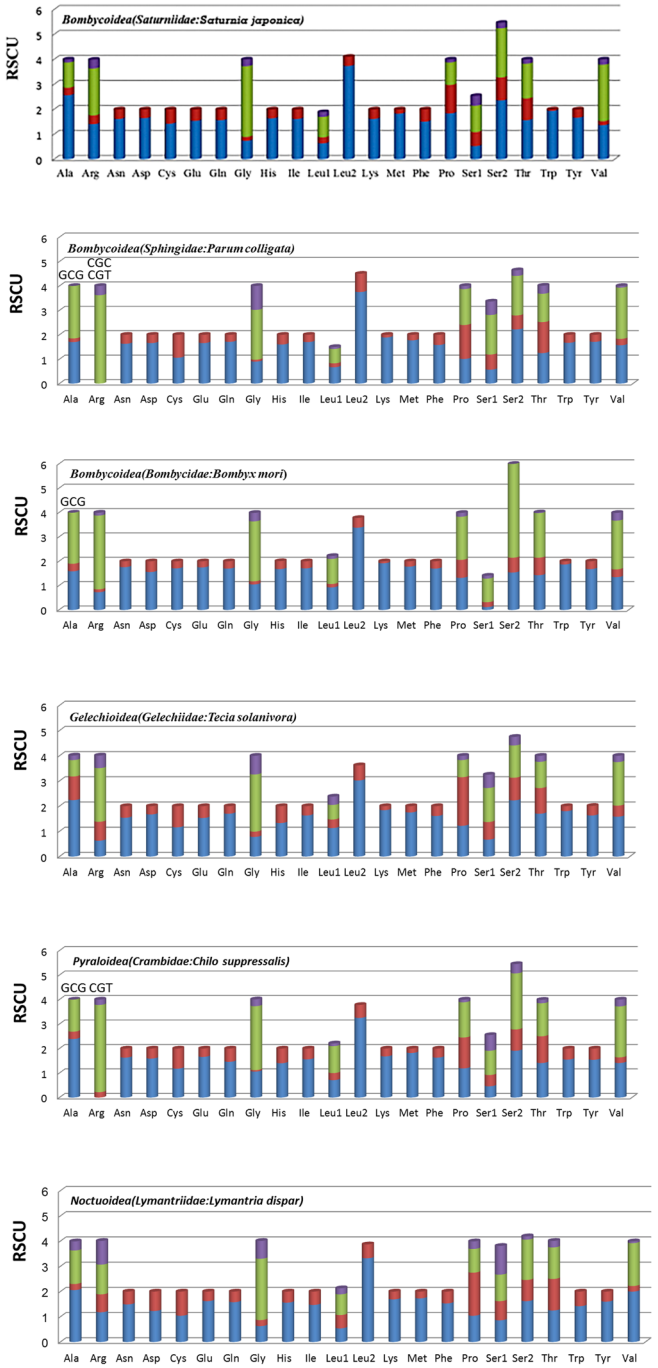


Fig. 8 The Relative Synonymous Codon Usage (RSCU) of the mitogenome of eight superfamilies in the Lepidoptera. Codon families are plotted on the X-axis. Codons indicated above the bar are not present in the mitogenome

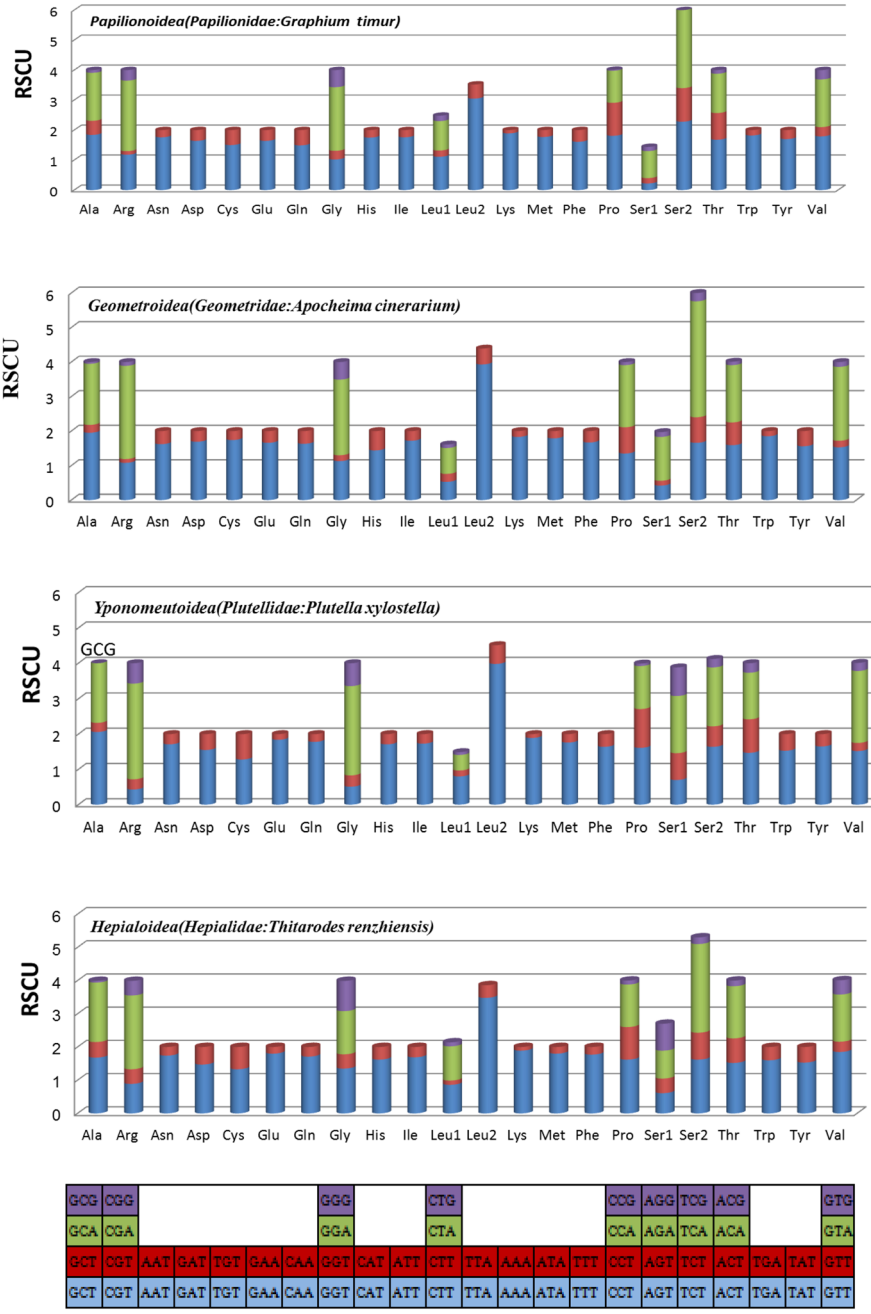


Fig. 8 (continued)

Table 5 Composition and skewness in different Lepidopteran mitogenomes

Species	Size(kb)	A%	G%	T%	C%	A + T%	AT skewness	GC skewness
PCG								
S. japonica	11.235	38.83	8.31	40.33	12.52	79.16	− 0.019	− 0.202
A. assama	11.211	38.85	8.44	39.94	12.77	78.79	− 0.014	− 0.204
P. colligata	11.172	40.22	8.43	39.37	11.99	79.58	0.011	− 0.174
L. dispar	11. 236	39.68	8.45	38.15	13.71	77.84	0.02	− 0.238
A. ipsilon	11. 211	39.71	8.46	40.09	11.73	79.81	− 0.005	− 0.162
P. flavescens	11. 211	39.42	8.89	39.55	12.14	78.97	− 0.002	− 0.154
H. cunea	11. 205	39.99	8.35	38.6	13.06	78.59	0.018	− 0.22
A. cinerarium	11. 225	40.63	8.78	38.19	12.39	78.82	0.031	− 0.17
P. atrilineata	11. 203	40.23	8.59	38.87	12.31	79.1	0.017	− 0.178
C. suppressalis	11.230	40.42	8.16	38.48	12.95	78.9	0.025	− 0.227
E. junctalis	11. 207	39.31	8.67	40.2	11.81	79.51	− 0.011	− 0.153
T. solanivora	11.175	37.85	9.3	38.52	14.33	76.38	− 0.009	− 0.213
P. suzukiella	11.200	39.07	8.44	40.64	11.85	79.71	− 0.02	− 0.168
P. nomion	11.198	39.55	8.21	40.65	11.59	80.2	− 0.014	− 0.171
L. botrana	11.154	39.83	8.61	38.73	12.83	78.56	0.014	− 0.197
G. timur	11.213	39.23	8.68	39.55	12.54	78.78	− 0.004	− 0.182
T. maculata	11.176	38.62	8.32	41.03	12.03	79.65	− 0.03	− 0.182
C. pomona	11.203	39.05	8.29	41.03	11.62	80.09	− 0.025	− 0.167
C. benjaminii	11.153	39.44	8.23	39.74	12.59	79.18	− 0.004	− 0.209
T. renzhiensis	11.189	40.23	8.43	38.81	12.53	79.04	0.018	− 0.196
L. malifoliella	11.163	41.74	7.92	38.99	11.36	80.72	0.034	− 0.178
tRNA								
S. japonica	1477	40.35	7.85	41.44	10.36	81.79	− 0.013	− 0.138
A. assama	1466	39.97	8.12	40.59	11.32	80.56	− 0.008	− 0.165
P. colligata	1475	41.02	8	40.34	10.64	81.36	0.008	− 0.142
L. dispar	1469	41.66	7.96	39.35	11.03	81.01	0.029	− 0.161
A. ipsilon	1477	41.23	8.12	40.42	10.22	81.65	0.01	− 0.114
P. flavescens	1485	41.62	7.81	40.61	9.97	82.22	0.012	− 0.121
H. cunea	1474	41.86	7.87	39.89	10.38	81.75	0.024	− 0.138
A. cinerarium	1483	42.01	8.02	39.45	10.52	81.46	0.031	− 0.135
P. atrilineata	1476	41.4	8.2	40.04	10.37	81.44	0.017	− 0.117
C. suppressalis	1482	40.89	7.89	40.89	10.32	81.78	0	− 0.133
E. junctalis	1480	41.49	8.24	40.07	10.2	81.55	0.017	− 0.106
T. solanivora	1477	41.16	8.46	39.54	10.83	80.7	0.02	− 0.123
P. suzukiella	1490	41.21	7.99	40.47	10.34	81.68	0.009	− 0.128
P. nomion	1447	40.43	8.29	40.57	10.71	81	− 0.002	− 0.127
L. botrana	1453	40.81	8.4	39.78	11.01	80.59	0.013	− 0.135
G. timur	1448	40.33	8.01	41.23	10.43	81.56	− 0.011	− 0.131
T. maculata	1454	40.51	7.84	41.06	10.59	81.57	− 0.007	− 0.149
C. pomona	1454	39.96	8.25	41.06	10.73	81.02	− 0.014	− 0.13
C. benjaminii	1469	41.25	7.83	40.44	10.48	81.69	0.01	− 0.145

Table 5 (continued)

Species	Size(kb)	A%	G%	T%	C%	A + T%	AT skewness	GC skewness
T. renzhiensis	1473	43.72	7.13	39.71	9.44	83.44	0.048	− 0.139
L. malifoliella	1488	41.4	6.79	42.27	9.54	83.67	− 0.01	− 0.169
rRNA								
S. japonica	2179	40.8	4.96	43.92	10.33	84.72	− 0.037	− 0.351
A. assama	2150	40.98	4.98	43.4	10.65	84.37	− 0.029	− 0.363
P. colligata	2090	42.92	4.83	42.34	9.9	85.26	0.007	− 0.344
L. dispar	2150	42.79	4.79	41.81	10.6	84.6	0.012	− 0.378
A. ipsilon	2162	41.58	5	43.57	9.85	85.15	− 0.023	− 0.327
P. flavescens	2198	41.31	4.73	44.04	9.92	85.35	− 0.032	− 0.354
H. cunea	2234	42.08	4.92	42.75	10.25	84.83	− 0.008	− 0.351
A. cinerarium	2179	43.97	4.77	41.17	10.1	85.13	0.033	− 0.358
P. atrilineata	2203	42.85	4.58	43.08	9.49	85.93	− 0.003	− 0.348
C. suppressalis	2171	41.27	4.97	43.67	10.09	84.94	− 0.028	− 0.339
E. junctalis	2123	40.56	4.99	44.42	10.03	84.97	− 0.045	− 0.335
T. solanivora	2092	39.82	5.21	43.79	11.19	83.6	− 0.047	− 0.364
P. suzukiella	2142	39.59	4.9	45.94	9.57	85.53	− 0.074	− 0.323
P. nomion	2145	41.72	5.03	42.94	10.3	84.66	− 0.014	− 0.343
L. botrana	2149	40.95	5.03	43.93	10.1	84.88	− 0.035	− 0.335
G. timur	2112	41.62	5.07	43.13	10.18	84.75	− 0.018	− 0.335
T. maculata	2109	39.73	4.84	45.66	9.77	85.4	− 0.069	− 0.338
C. pomona	2111	40.41	5.07	44.77	9.76	85.17	− 0.051	− 0.316
C. benjaminii	2132	41.7	4.88	43.76	9.66	85.46	− 0.024	− 0.329
T. renzhiensis	2114	41.2	5.16	44.18	9.46	85.38	− 0.035	− 0.294
L. malifoliella	2121	41.49	4.71	44.6	9.19	86.09	− 0.036	− 0.322
A + T-rich region								
S. japonica	332	42.17	2.11	49.7	6.02	91.87	− 0.082	− 0.481
A. assama	332	40.96	2.11	49.7	7.23	90.66	− 0.096	− 0.548
P.colligata	358	43.58	1.68	51.96	2.79	95.53	− 0.088	− 0.25
L. dispar	435	45.29	1.61	50.8	2.3	96.09	− 0.057	− 0.176
A. ipsilon	332	46.08	1.51	48.8	3.61	94.88	− 0.029	− 0.412
P. flavescens	541	42.14	2.22	49.72	5.91	91.87	− 0.082	− 0.455
H. cunea	357	45.66	1.12	49.3	3.92	94.96	− 0.038	− 0.556
A. cinerarium	625	47.2	1.92	48.64	2.24	95.84	− 0.015	− 0.077
P.atrilineata	457	40.7	0.66	57.55	1.09	98.25	− 0.171	− 0.25
C. suppressalis	348	42.24	0.29	53.16	4.31	95.4	− 0.114	− 0.875
E. junctalis	351	43.87	2.28	50.43	3.42	94.3	− 0.069	− 0.2
T. solanivora	332	43.07	2.71	48.19	6.02	91.27	− 0.056	− 0.379
P. suzukiella	369	50.68	0.54	46.34	2.44	97.02	0.045	− 0.636
P. nomion	483	44.31	2.69	48.65	4.35	92.96	− 0.047	− 0.235
L. botrana	286	40.21	2.45	51.4	5.94	91.61	− 0.122	− 0.417
G. timur	403	46.9	1.49	48.88	2.73	95.78	− 0.021	− 0.294
T. maculata	382	44.5	2.88	48.69	3.93	93.19	− 0.045	− 0.154

Table 5 (continued)

Species	Size(kb)	A%	G%	T%	C%	A + T%	AT skewness	GC skewness
C. pomona	313	46.65	0.32	50.48	2.56	97.12	− 0.039	− 0.778
C. benjaminii	293	46.42	3.07	45.73	4.78	92.15	0.007	− 0.217
T. renzhiensis	1367	45.06	4.68	45.5	4.75	90.56	− 0.005	− 0.008
L. malifoliella	733	46.25	0.95	49.11	3.68	95.36	− 0.03	− 0.588

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