Mol Biol Rep (2010) 37:3431–3438
DOI 10.1007/s11033-009-9934-3

The complete mitochondrial genome of the yellow coaster, *Acraea issoria* (Lepidoptera: Nymphalidae: Heliconiinae: Acraeini): sequence, gene organization and a unique tRNA translocation event

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Received: 17 September 2009 / Accepted: 16 November 2009 / Published online: 29 November 2009

Abstract In this paper, the complete mitochondrial genome of *Acraea issoria* (Lepidoptera: Nymphalidae: Heliconiinae: Acraeini) is reported; a circular molecule of 15,245 bp in size. For *A. issoria*, genes are arranged in the same order and orientation as the complete sequenced mitochondrial genomes of the other lepidopteran species, except for the presence of an extra copy of $tRNA^{Ile(AUR)b}$ in the control region. All protein-coding genes of *A. issoria* mitogenome start with a typical ATN codon and terminate in the common stop codon TAA, except that COI gene uses TTG as its initial codon and terminates in a single T residue. All tRNA genes possess the typical clover leaf secondary structure except for $tRNA^{Ser(AGN)}$, which has a simple loop with the absence of the DHU stem. The sequence, organization and other features including nucleotide composition and codon usage of this mitochondrial genome were also reported and compared with those of other sequenced lepidopterans mitochondrial genomes. There are some short microsatellite-like repeat regions (e.g., $(TA)_9$, polyA and polyT) scattered in the control region, however, the conspicuous macro-repeats units commonly found in other insect species are absent.

Keywords Mitochondrial genome · Lepidoptera · Nymphalidae · Heliconiinae · *Acraea issoria*

Introduction

Insect mitochondrial DNA (mtDNA) consists of a circular molecule of 13–19 kb in size, with 13 protein-coding genes (PCGs), two ribosomal RNA genes and 22 tRNA genes [1, 2]. Additionally, it contains a major non-coding area, i.e., the control region or the A + T-rich region, which regulates the transcription and replication of the mitochondrial genome [2, 3]. Though gene content of the insect mitochondrial genome (mitogenome) is relatively conserved, there are a few of exceptions. For example, *Coreana rapaelis* (Lepidoptera: Lycaenidae) has an extra $tRNA^{Ser(AGN)}$; *Bombyx mandaria* (Lepidoptera: Bombycidae) [4] possesses two extra tRNA-like genes: the $tRNA^{Ser(TGA)}$-like and the $tRNA^{Ile(TAT)}$-like; both the dipterans *Chrysomya chloropyga* and *C. megacephala* have an extra $tRNA^{Ile}$.

Mainly because of its maternal inheritance, lack of recombination and an accelerated mutation rate compared to those of the nuclear DNA, the use of mtDNA has become popular in the studies of phylogenetics, comparative and evolutionary genomics, population genetics and molecular evolution among various animal taxa [5, 6]. So far, more than 160 complete or near complete mitogenome

Electronic supplementary material The online version of this article (doi:10.1007/s11033-009-9934-3) contains supplementary material, which is available to authorized users.

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Table 1 Lepidopteran mitogenomes used in this study

Species	Family	Accession number
Coreana raphaelis	Lycaenidae	NC_007976
Artogeia melete	Pieridae	NC_010568
Papilio xuthus	Papilionidae	EF621724
Bombyx mandarina	Bombycidae	NC_003395
Bombyx mori	Bombycidae	NC_002355
Adoxophyes honmai	Tortricidae	NC_008141
Phthonandria atrilineata	Geometridae	EU569764
Ochrogaster lunifer	Notodontidae	NC_011128
Manaduca sexta	Sphingidae	NC_010266
Antheraea pernyi	Saturniidae	AY242996
Saturnia boisduvalii	Saturniidae	NC_010613
Eriogyna pyretorum	Saturniidae	NC_012727
Ostrinia nubilalis	Pyraloidea	AF442957
Ostrinia furnacalis	Pyraloidea	NC_003368

sequences have been reported from insects. There are 14 species complete or near complete sequences from the Lepidoptera (Table 1). The order Lepidoptera, which includes insects such as the butterflies and moths, has more than 160,000 species all around the world. Despite of their huge taxonomic diversity, the existing information about their mtDNA, especially the complete genomes of mtDNA is still limited. So, the further insights into lepidopteran phylogeny and taxonomy await more related sequences to be determined.

The taxonomic status and the phylogenetic position of *Acraea* still remain as a controversial issue [7–10]. Chinese scholars such as Chou assigned *Acraea* to a family level group, Acraeidae, based on morphological characters such as the shape of their relatively long forewing, incurved humeral vein, asymmetric claws, etc. [7]. In contrast South American and African scholars assigned *Acraea* to a tribal level (Acraeini) within the subfamily Heliconiinae (Nymphalidae). Penz and Peggie [8] proposed the Acraeini appeared as the sister group of Heliconiini + (Vagrantini + Argynnini), based on the morphological characters of early immatures and adults. Freitas and Brown [9] found the same results from studies of larval, pupal and adult characters such as the wing veins and scales, shape of prothoracic legs, male and female genitalia and abdomen, etc. Based on a combined analysis of the genes cytochrome oxidase I (COI), elongation factor 1-α (EF-1α) and wingless (wgl), Silva-Brandão et al. [10] reconstructed the phylogenetic trees of the tribe Acraeini. These results showed that the Acraeini is sister to Heliconiini + Cethosiini based on bayesian inference, whereas, the Acraeini is the sister of Vagrantini + Argynnini (containing *Pardopsis*) based on parsimony analysis.

In this paper, we tried to amplify and sequence the complete mitogenome of *A. issoria* (Lepidoptera: Heliconiinae), and to compare its sequence with those of other lepidopterans available from GenBank. We aim to supply more molecular information for further studies of the lepidopteran phylogeny and to clarify the taxonomic status of the Acraeini.

Materials and methods

Specimen collection

Adult individuals of *A. issoria* were collected in Huangshan Mountains, Anhui Province, China. After collection, the fresh materials were preserved in 100% ethanol immediately and stored at −20°C before the DNA extraction.

DNA extraction, PCR amplification and sequencing

Whole genomic DNA was extracted from thoracic muscle tissue with the DNeasy Tissue kit (Qiagen). Some universal PCR primers for short fragment amplifications of 12S, COI, CYTB, ND1 and COII genes were synthesized after Simon et al. [11], Simons and Weller [12], and Caterino and Sperling [13]. Long primers and some short ones including ND2, ND4, COIII, and ND5 were designed by the multiple sequence alignments of the complete mitochondrial genomes of all the Lepidopterans available, using ClustalX1.8 [14] and Primer Premier 5.0 softwares. The entire genome of *A. issoria* was amplified in nine fragments (COI–COII, COII–COIII, COIII–ND5, ND5–ND4, ND4–CYTB, CYTB–ND1, ND1–12S, 12S–ND2, ND2–COI) using long PCR technique, which is performed using TaKaRa LA Taq polymerase with the following cycling parameters: 95°C for 5 min; 30 cycles of 95°C for 50 s, 50°C for 50 s, 68°C for 2 min and 30 s; and a final extension step of 68° for 10 min. The PCR products were detected via electrophoresis in 1.2% agarose gel, purified using the 3S Spin PCR Product Purification Kit and sequenced directly with ABI–377 automatic DNA sequencer; all the amplified products were sequenced directly except for the COIII-ND5 and 12S-ND2, which were sequenced after cloning; for each long PCR product, the full, double stranded sequence was determined by primer walking.

Analysis and annotation

Raw sequence files were proof read and assembled in BioEdit version 7.0 [15]; PCGs and ribosomal RNA genes were identified by sequences using ClustalX1.8 software

[14] and the NCBI Internet BLAST search function; transfer RNA gene analysis was conducted using tRNA-scan-SE software v.1.21 [16]; the putatived tRNAs not found by tRNAscan-SE were confirmed by sequence comparison of *A. issoria* with other lepidopterans; nucleotide composition and codon usage were calculated in MEGA 3.0 software [17]. The sequence data have been deposited into the GenBank database under the accession number GQ376195.

Results and discussion

Genome organization and structure

The mitogenome of *A. issoria* is a circular molecule 15,245 bp long and consists of 13 PCGs, two ribosomal RNA genes for the small and large subunits (srRNA and lrRNA), and 22 transfer RNA genes, and the same as the typical animal mitogenome (Fig. 1) with the exception of a extra $tRNA^{Ile(AUR)}$ copy. The mtDNA genome of *A. issoria* includes 13 intergenic spacers, ranging from 1 to 430 bp (517 bp in total), of which only two spacers span longer than 10 bp (Table 2); additionally, a total of 63 bp overlapped nucleotides are scattered all over the genome, and

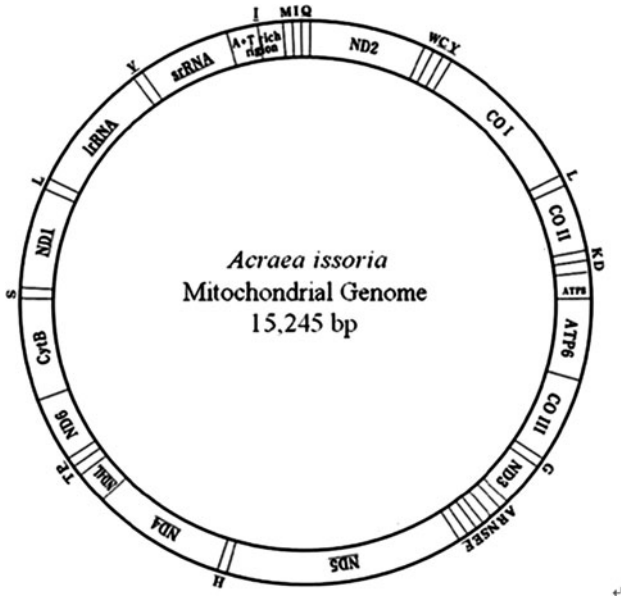

Fig. 1 Map of the mitochondrial genome of *A. issoria*. The abbreviations for the genes are as follows: ATP6, ATP8, ATP synthase subunits 6 and 8 genes; CYTB, cytochrome oxidase b gene; COI–COIII, cytochrome oxidase c subunit 1–3 genes; ND1–6, ND4L, NADH dehydrogenase subunits 1–6 and 4L. Gene names are the standard abbreviations used in this paper; tRNA genes are denoted as one-letter symbol according to the IUPAC-IUB single letter amino acid codes. Gene names that are not underlined indicate the direction of transcription clockwise, while the underline indicates counter clockwise

among which the longest is 35 bp long located between COII and $tRNA^{Lys}$, the seven nucleotides (ATGATAA) is overlapped between ATP8 and ATP6 (Table 2). This feature is common to all lepidopteran mtDNA genomes sequenced to date and functions in forming a hairpin loop structure for posttranslational modification [18, 19].

The nucleotide composition bias of the mitogenome of *A. issoria* is 79.7% A + T, made up of 78.0% in the PCGs, 83.8% in the ribosomal RNA genes, 82.3% in the transfer RNA genes and 96% in the control region, respectively. This is similar to the nucleotide composition biases observed in other insect mitochondrial genomes, which ranges from 65.6% in *Reticulitermes* (Isoptera) [20, 21] to 89.3% in *Apis* (Hymenoptera) [22]. The nucleotide frequency of the PCGs on the majority (J strand) and the minority strand (N strand) is T > A > C > G and A > T > C > G, respectively, with a strong AT bias. In addition, the CG and AT skews both on the J and N strands for each mitochondrial genome were thought as a measure of the compositional asymmetry [23] (Table 3), and in this study, the PCGs on the J strand display significantly a AT skew (−0.140) and a GC skew (0.176), compared to that of other lepidopterans (AT skews are from −0.161 to −0.045, GC skews are from 0.071 to 0.239). On the contrary, PCGs on the N strand lack notable AT and GC skews. The nucleotide bias for AT was also reflected in the condon usage and the relative synonymous codon usage (RSCU) (see Table 4 in Supplementary Material), that is codons for A or T (A = 39.75%, T = 50.11%) strongly overpasses C or G (C = 6.40%, G = 3.74%) in the third codon position. The most frequent amino acids in the PCGs of *A. issoria* are Leucine (14.42%), Isoleucine (12.24%), Phenylalanine (10.58%) and Serine (8.34%).

Gene order

Among the 11 known complete mitogenome sequences from lepidopterans, their orientation and gene order exist four basic arrangement patterns and all differ from the ancestral gene order of insects in the location of $tRNA^{Met}$ [2]. In all Lepidoptera sequenced to date $tRNA^{Met}$ is located between the control region and $tRNA^{Ile}$, giving the derived control region (CR)-Met (M)-Ile (I)-Glu (Q) arrangement instead of that of the insect ground plan CR-I-Q-M. This phenomenon was first reported in lycaenids and noctuids by Taylor et al. [24] and has now been found in five lepidopteran superfamilies (Papilionoidea, Noctuodiea, Tortricoidea, Pyraloidea, and Bombyciodea) [25] (Fig. 2): *Bombyx mandarina*, *Bombyx mori*, *Saturnia boisduvalii*, *Adoxophyes honmai*, *Phthonandria atrilineata*, *Ochrogaster lunifer*, *Antheraea pernyi*, *Manduca sexta*, *Artogeia melete* and *Eriogyna pyretorum* share the same patter as Fig. 2-1; *Bombyx mandaria* [4] has the pattern shown as

Table 2 Organization of the *A. issoria* mitochondrial genome

Gene	Direction	Nucleotide no.	Size(bp)	Intergenic nucleotides	Start code	Stop code
$tRNA^{Met}$	F	1–68	68			
$tRNA^{Ile(AUR)}$	F	69–134	66	0		
$tRNA^{Gln}$	R	132–200	69	−3		
ND2	F	252–1265	1014	51	ATT	TAA
$tRNA^{Trp}$	F	1270–1335	66	4		
$tRNA^{Cys}$	R	1328–1392	65	−8		
$tRNA^{Tyr}$	R	1394–1459	66	1		
COI	F	1460–2991	1532	0	TTG	T–tRNA
$tRNA^{Leu(UUR)}$	F	2992–3058	67	0		
COII	F	3059–3769	711	0	ATG	TAA
$tRNA^{Lys}$	F	3735–3805	71	−35		
$tRNA^{Asp}$	F	3805–3870	66	−1		
ATP8	F	3871–4032	162	0	ATG	TAA
ATP6	F	4026–4703	678	−7	ATG	TAA
COIII	F	4703–5491	789	−1	ATG	TAA
$tRNA^{Gly}$	F	5493–5558	65	2		
ND3	F	5559–5912	354	0	ATT	TAA
$tRNA^{Ala}$	F	5917–5981	65	4		
$tRNA^{Arg}$	F	5984–6046	63	2		
$tRNA^{Asn}$	F	6047–6112	66	0		
$tRNA^{Ser(AGN)}$	F	6111–6171	61	−2		
$tRNA^{Glu}$	F	6173–6238	66	1		
$tRNA^{Phe}$	R	6237–6301	65	−2		
ND5	R	6301–8025	1725	−1	ATA	TAA
$tRNA^{His}$	R	8034–8099	66	8		
ND4	R	8104–9444	1341	4	ATG	TAA
ND4L	R	9445–9726	282	0	ATG	TAA
$tRNA^{Thr}$	F	9729–9791	63	2		
$tRNA^{Pro}$	R	9792–9856	65	0		
ND6	F	9862–10389	528	5	ATA	TAA
Cytb	F	10393–11541	1149	3	ATG	TAA
$tRNA^{Ser(UCN)}$	F	11540–11608	69	−2		
ND1	R	11607–12563	957	−1	ATG	TAA
$tRNA^{Leu(CUN)}$	R	12564–12631	68	0		
lrRNA	R	12632–13962	1331	0		
$tRNA^{Val}$	R	13963–14027	65	0		
srRNA	R	14028–14815	788	0		
D-loop	F	14816–15245	430			
$tRNA^{Ile(AUR)b}$	R	14997–15079	83			

tRNA abbreviations follow the IU-PAC-IUB three letter code. For other abbreviations see legend for Fig. 1

[b] Extra copy

Fig. 2-2, in which the two extra tRNA-like genes, the $tRNA^{Ser(TGA)}$-like and the $tRNA^{Ile(TAT)}$-like between the control region and $tRNA^{Met}$ and between the $tRNA^{Gln}$ and ND2, respectively; *Coreana raphaelis* has the pattern as Fig. 2-3 in which an extra $tRNA^{Ser(AGN)}$ is located between $tRNA^{Ser(AGN)}$ and $tRNA^{Glu;}$ *A. issoria* is the pattern shown as Fig. 2-4 in which an extra $tRNA^{Ile}$ is located in the control region.

Protein-coding genes

Except for COI, 12 out of 13 PCGs in *A. issoria* use standard ATN start codons (ATA for ND5 and ND6; ATT for ND2 and ND3; ATG for COII, ATP8, ATP6, COIII, ND4, ND4L, CYTB and ND1). However, the COI gene is generally use non-canonical initial codon for the reason that no regular start codon is available after the last stop

Table 3 Comparison of mitochondrial proteins for Lepidoptera

Species	Size (bp)		AT (%)		AT-skew		CG-skew	
	J strand	N strand	J strand	N strand	J strand	N strand	J strand	N strand
Bombyx mori	6881	4296	78.5	81.3	−0.045	0.269	0.131	0.326
Bombyx mandarina	7004	4296	78.7	81.1	−0.050	0.268	0.132	0.330
Antheraea pernyi	6900	4327	77.0	81.0	−0.153	0.185	0.148	0.309
Ostrinia furnacalis	6886	4307	78.2	81.3	−0.079	0.215	0.110	0.312
Ostrinia nubilalis	6883	4308	78.0	81.0	−0.077	0.215	0.105	0.305
Adoxophyes honmai	6871	4377	77.1	80.6	−0.115	0.199	0.123	0.299
Manduca sexta	6903	4282	79.3	82.0	−0.112	0.193	0.087	0.311
Saturnia boisduvalii	6847	4328	78.0	80.8	−0.151	0.186	0.127	0.330
Eriogyna pyretorum	6897	4329	78.2	81.3	−0.161	0.171	0.128	0.294
Phthonandria atrilineata	6893	4310	78.2	80.6	−0.100	0.199	0.105	0.306
Ochrogaster lunifer	6915	4452	74.5	77.9	−0.087	0.214	0.239	0.423
Coreana raphaelis	6862	4289	80.3	83.4	−0.141	0.115	0.071	0.273
Artogeia melete	6890	4290	77.7	79.8	−0.097	0.211	0.148	0.327
Acraea issoria	6917	4305	76.7	80.1	−0.140	0.161	0.176	0.273
Avg.	6896.3	4321.1	77.89	80.87	−0.108	0.2001	0.1306	0.315

J strand (majority strand) encodes COI-III, CYTB, ATP8, ATP6, ND2, ND3 and ND6

N strand (minority strand) encodes ND4, ND4L, ND5 and ND1

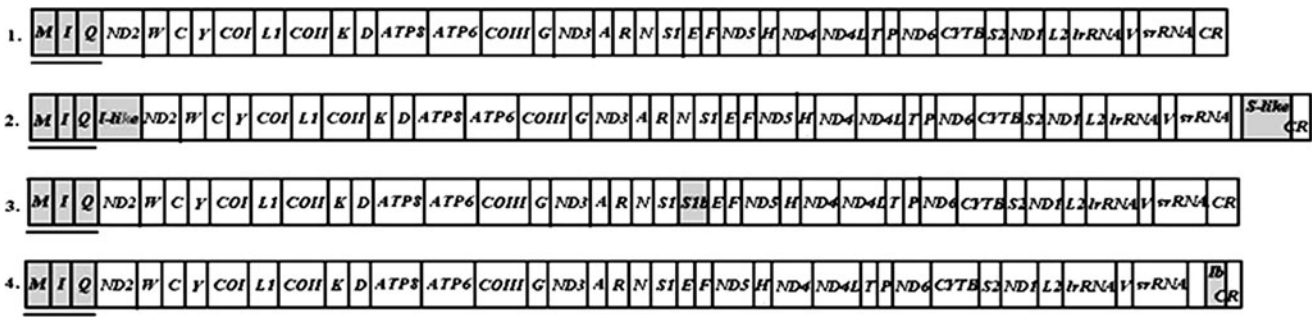

Fig. 2 Compare the gene arrangements of the Lepidoptera mtDNA (*Shadows* indicate differences existing among the lepidopteran species. *S1*: tRNA$^{Ser(AGN)}$, *S2*: tRNA$^{Ser(UCN)}$, *L1*: tRNA$^{Leu(UUR)}$, *L2*: tRNA$^{Leu(CUN)}$, *b*: extra copy)

codon upstreaming the COI open reading frame [26]. All lepidopteran species sequenced to date use CGA (R) as the initial site for COI which is common in insects [19]. In contrast, Kim et al. [18] considered the tetranucleotide TTAG abutting to CAG as the initiation site for COI in *C. raphaelis*; Clary and Wolstenholme [27] and de Bruijn [28] proposed that the start codon of COI was tetranucleotides ATAA or ATTA; moreover, some hexanucleotides, such as TATTAG in *Ostrinia nubilalis* and *Ostrinia furnicalis* [29], TTTTAG in the *Bombyx mori*, TATCTA in *Penaeus monodon* [30], ATTTAA in *Anopheles gambiae* [31], *Anopheles quadrimaculatus* [32] and *Ceratitis capitata* [33], were also putatived to be the start codon for COI. In this study, a typical ATN initiator for COI in *A. issoria* is not found in the start site either. Furthermore, TTG as the initiation codon for COI has been reported in the invertebrate and some insects including *Anopheles quadrimaculatus* [32], *Pyrocoelia rufa* [34] and *Caligula boisdnvalii* [35], and thus it is reasonable to consider that TTG codon lying in the beginning region of COI to be the possible initiation codon for COI of *A. issoria*.

12 PCGs have the common stop codon (TAA), while COI terminates in a single T residue in this study. Incomplete stop codons would produce functional stop-codons after polycistronic transcript cleavage and polyadenylation mechanisms [36] and have been found in most insect mitogenomes including all lepidopteran species sequenced to date [18, 26, 29, 37].

Transfer RNAs and ribosomal RNAs

A. issoria has the typical 22 tRNAs set and an extra tRNA$^{Ile(AUR)b}$ located in the control region (see Fig. 3 in Supplementary Material). All of them possess a complete

clover leaf secondary structure, except that $tRNA^{Ser(AGN)}$ lacks DHU stem. In general, the lack of DHU stem in $tRNA^{Ser}$ is a common feature in metazoan mtDNAs [38], but there are exceptions such as the tea tortrix *Adoxophyes honmai* (Tortricidae) [26], or the screamer louse, *Bothriometopus* (Philopteridae) [21] all of whose tRNAs have a complete clover leaf structure. The $tRNA^{Ile(AUR)b}$ structure of *A. issoria* differs substantively from the gene copy in the consensus lepidopteran position. It has three extra nucleotides (ATA) in the DHU loop, ten nucleotides (TAAAAAATAT) in the anticodon loop and one nucleotide (A) in the extra loop, respectively, in total 83 bp in size. If the additional nucleotides were removed by posttranscriptional modification, it would still form the same gene ($tRNA^{Ile}$) with a standard clover leaf structure. Unlike other insects with duplicated $tRNA^{Ile}$ genes, such as the dipterans *Chrysomya chloropyga* [39] and *Chrysomya megacephala* [40] there is limited sequence similarity between the two copies of $tRNA^{Ile}$ in *A. issoria*.

The tRNA acceptor stems are all 7nt whereas the DHU loops (4-9nt) and TΨC loops (3-8nt) are more variable. 15 tRNA genes show 22 pair mismatches in their stems, including nine pairs in the DHU stems, eight pairs in the amino acid acceptor stems, three pairs in the TΨC stems and two pairs in the anticodon stems. The mismatched bases are mainly G·U, U·G or U·U, with the exception represented by $tRNA^{Ile}$ which exhibits G·A and C·U mismatches. These mismatches are corrected through RNA-editing mechanisms [38].

LrRNA and srRNA genes are located between $tRNA^{Leu}$ and $tRNA^{Val}$, and between $tRNA^{Val}$ and A + T-rich region, respectively. They are 1331 and 788 bp in length, respectively. The lengths of the two rRNAs are similar to that of other lepidopterans.

Non-coding regions

The mtDNA genome of *A. issoria* includes 13 intergenic spacers, ranging from 1 to 430 bp (517 bp in total). There are only two spacers longer than 10 bp (Table 2): spacer 1 is 51 bp long located between $tRNA^{Gln}$ and ND2, whereas, spacer 2 is 430 bp located between srRNA and $tRNA^{Met}$. In addition, the other 11 small intergenic spacers (<10 bp) are dispersed throughout the whole genome, totaling 36 bp in length. Non-coding spacer 1 is present in all of the lepidopteran mitogenomes studied to date, but absent in all non-lepidopteran insects. The A + T content of this spacer are 88.2%, which is lower than those of other sequenced lepidopterans, in which a high A + T content (93–100%) was reported [29, 37]. This spacer's location is fixed among the lepidopterans, while their sequences are highly diverged even among two congeneric species examined (*Ostrinia furnicalis* and *Ostrinia nubilalis*; *Bombyx mori* and *Bombyx mandarina*) [25]. This spacer is a constant molecular signature of lepidopteran mtDNA; however, it is necessary to test this with a wider taxon sample. Spacer 2 comprises the control region which regulates the transcription and replication of the mitogenome [41], and includes the O_N (origin of minority or light strand replication) identifiable by the motif ATAGA located 14 bp downstream from srRNA, followed by an 18 bp polyT. This O_N motif is conserved among all lepidopteron, however the polyT stretch varies in length ranging from 18 to 22 bp. Additionally, the CR includes multiple short microsatellite-like repeat regions (e.g., $(TA)_9$, polyA and polyT). The conspicuous macro-repeat units (50+ bp in size) commonly found in other insect species are absent in the control region. The microsatellite-like TG $(TA)_9$ element preceded by the ATTTA motif is present in the 3′ flanking region of spacer 2. Similar motifs, $ATTTA(AT)_9$, $ATTTA(AT)_{10}$, $ATTTA(TA)_8$, $ATTTA(AT)_7(TA)_3$, $ATTTA(AT)_7$ and $ATTTA(AT)_{11}$, are found in *Bombyx mandarina*, *Bombyx mori*, *Manaduea sexta*, *Ochrogaster lunifer*, *Coreana raphaelis* and *Artogeia melete*, respectively.

Conclusion

The mitochondrial genome of *A. issoria* is 15,245 bp in size. It has a relatively low AT content (79.7%) compared to other lepidopteran species. The orientation and gene order of the *A. issoria* is similar to those of other lepidopteran species, except for the presence of an extra copy of $tRNA^{Ile(AUR)b}$ in the control region. Majority-strand PCGs display significant AT and GC skews. On the contrary, minority-strand PCGs lack notable AT and GC skews. The nucleotide bias for AT were also reflected in the condon usage and the relative synonymous codon usage. Codons with A or T in the third codon position (A = 39.75%, T = 50.11%) greatly exceeds those with a C or G (C = 6.40%, G = 3.74%). The complete mitogenome of *A. issoria* reported here is expected to supply more molecular information for further studies of the lepidopteran phylogeny and for analyses on the taxonomic status of the Acraeini.

Acknowledgments This work was supported by the Scientific and Technologic Funds for the Outstanding Youth in Anhui Province (Grant No. 08040106811), the special funds for the outstanding college scholars and the "Key Laboratory of the Conservation and Utilization of Important Biological Resources" in Anhui Province, and partially by Supported by a grant (No. O529YX5105) from the Key Laboratory of the Zoological Systematics and Evolution of the Chinese Academy of Sciences, Public Welfare Project from the Ministry of Agriculture, China (Grant No. 200803006). We also thank Dr. Gai Yonghua (Nanjing Institute of Geology and Paleontology, Chinese Academy of Sciences), Liu Gang (College of Life Sciences, Anhui Normal University), Yu Fang, Yuan Feng, Chen FuQiang and

Wang Chen (Institute of Zoology, Chinese Academy of Sciences) for their comments on the manuscript and kindly help in sequence analysis.

References

1. Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. Int Rev Cyt 141:173–216
2. Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Res 27:1767–1780
3. Taanman JW (1999) The mitochondrial genome: structure, transcription, translation and replication. Biochim Biophys Acta 1410:103–123
4. Pan MH, Yu QY, Xia YL, Dai FY, Liu YQ, Lu C, Zhang Z, Xiang ZH (2008) The organization of the Chinese *Bombyx mandarina* mitochondrial genome. Science 8:751–759
5. Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondrial. Mol Ecol 13:729–744
6. Brown WM, George M, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76: 1967–1971
7. Chou I (1998) Classification and Identification of Chinese butterflies. Henan Scientific and Technological Publishing House, Zhengzhou, pp 173–176
8. Penz CM, Peggie D (2003) Phylogenetic relationships among Heliconiinae genera based on morphology (Lepidoptera: Nymphalidae). Syst Entomol 28:451–479
9. Freitas AVL, Brown KS (2004) Phylogeny of the Nymphalidae (Lepidoptera). Syst Biol 53:363–383
10. Silva-Brandão KL, Wahlberg N, Francini RB, Azeredo-Espin AML, Brown KS Jr, Paluch M, Lees DC, Freitas AVL (2008) Phylogenetic relationships of butterflies of the tribe Acraeini (Lepidoptera, Nymphalidae, Heliconiinae) and the evolution of host plant use. Mol Phylogenet Evol 46:515–531
11. Simon C, Frati F, Bekenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial genesequences and a compilation of conserved polymerase chain-reaction primers. Ann Entomol Soc Am 87:651–701
12. Simons RB, Weller SJ (2001) Utility and evolution of cytochrome b in insects. Mol Phylogenet Evol 20:196–210
13. Caterino MS, Sperling FAH (1999) *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. Mol Phylogenet Evol 11:122–137
14. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25(24):4876–4882
15. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acid Symp Ser 41:95–98
16. Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964
17. Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163
18. Kim I, Lee EM, Seol KY, Yun EY, Lee YB, Hwang JS, Jin BR (2006) The mitochondrial genome of the Korean hairstreak, *Coreana raphaelis* (Lepidoptera: Lycaenidae). Insect Mol Biol 15:217–225
19. Fenn JD, Cameron SL, Whiting MF (2007) The complete mitochondrial genome of the Mormon cricket (*Anabrus simplex*: Tettigoniidae: Orthoptera) and an analysis of control region variability. Insect Mol Biol 16:239–252
20. Cameron SL, Whiting MF (2007) Mitochondrial genomic comparisons of the subterranean termites from the Genus *Reticulitermes* (Insecta: Isoptera: Rhinotermitidae). Genome 50:188–202
21. Cameron SL, Johnson KP, Whiting MF (2007) The mitochondrial genome of the screamer louse *Bothriometopus* (Phthiraptera: Ischnocera): effects of extensive gene rearrangements on the evolution of the genome as a whole. J Mol Evol 65:589–604
22. Cameron SL, Dowton M, Castro LR, Ruberu K, Whiting MF, Austin AD, Diement K, Stevens J (2008) The sequence of the mitochondrial genomes of two vespid wasps reveals a number of derived tRNA gene rearrangements. Genome 51:800–808
23. Perna NT, Kocher TD (1995) Patterns of nueleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J Mol Evol 41:353–358
24. Taylor MF, McKechnie SW, Pierce N, Kreitman M (1993) The lepidopteran mitochondrial control region: structure and evolution. Mol Biol Evol 10:1259–1272
25. Cameron SF, Whiting MF (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 408:112–123
26. Lee ES, Shin KS, Kim MS, Park H, Cho S, Kim CB (2006) The mitochondrial genome of the smaller tea tortix *Adoxophyes honmai* (Lepidoptera: Tortricidae). Gene 373:52–57
27. Clary DO, Wolstenholme DR (1983) Genes for cytochrome c oxidase subunit I, URF2 and three tRNAs in *Drosophila* mitochondrial DNA. Nucleic Acids Res 11:6859–6872
28. De Bruijn MHL (1983) *Drosophila melanogaster* mitochondrial DNA, a novel organization and genetic code. Nature 304: 234–241
29. Coates BS, Sumerford DV, Hellmich RL, Lewis LC (2005) Partial mitochondrial genome sequences of *Ostrinia nubilalis* and *Ostrinia furnicalis*. Int J Biol Sci 1:13–18
30. Wilson K, Cahill V, Ballment E, Benzie J (2000) The complete sequence of the mitochondrial genome of the crustacean *Penaeus monodon*: are Malacostracan crustaceans more closely related to insects than to Branchiopods. Mol Biol Evol 17(6):863–874
31. Beard CD, Hamm SM, Colllins FH (1993) The mitochondrial genome of the mosquito *Anopheles gambiae*: DNA sequence, genome organization, and comparisons with the mitochondrial sequences of other insect. Insect Mol Biol 2:103–124
32. Mitchell SE, Cockburn AF, Seawright JA (1993) The mitochondrial genome of *Anopheles quadrimaculatus* species A: complete nucleotide sequence and gene organization. Genome 36:1058–1073
33. Spanos L, Koutroumbras G, Kotsyfakis M, Louis C (2000) The mitochondrial genome of the Mediteranian fruit fly, *Ceratitis capitata*. Insect Mol Biol 9(2):139–144
34. Bae JS, Kim I, Sohn HD, Jin BR (2004) The mitochondrial genome of the firefly, *Pyrocoelia rufa*: complete DNA sequence, genome organization, and phylogenetic analysis with other insects. Mol Phylogenet Evol 32:978–985
35. Hong MY, Lee EM, Jo YH, Park HC, Kim SR, Hwang JS, Jin BR, Kang PD, Kim K, Han YS, Kim I (2008) Complete nucleotide sequence and organization of the mitogenome of the silk moth *Caligula boisduvalii* (Lepidoptera: Saturniidae) and comparison with other lepidopteran insects. Gene 413:49–57
36. Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature 290: 470–474
37. Yukuhiro K, Sezutsu H, Itoh M, Shimizu K, Banno Y (2002) Significant levels of sequence divergence and gene rearrangements have occurred between the mitochondrial genomes of the wild mulberry silk moth, *Bombyx mandarina*, and its close relative, the domesticated silk moth, *Bombyx mori*. Mol Biol Evol 19:1385–1389

38. Lavrov DV, Brown WM, Boore JL (2000) A novel type of RNA editing occurs in the mitochondrial tRNAs of the centipede *Lithobius forficatus*. Proc Natl Acad Sci USA 97:13738–13742
39. Junqueira ACM, Lessinger AC, Torres TT, da Silva FR, Vettore AL, Arruda P, Espin AMLA (2004) The mitochondrial genome of the blowfly *Chrysomya chloropyga* (Diptera: Calliphoridae). Gene 339:7–15
40. Lessinger AC, Azeredo-Espin AML (2000) Evolution and structural organisation of mitochondrial DNA control region of myiasis-causing flies. Med Vet Entomol 14:71–80
41. Clayton DA (1992) Transcription and replication of animal mitochondrial DNA. Int Rev Cyt 141:217–232