Mol Biol Rep (2011) 38:3757–3764
DOI 10.1007/s11033-010-0491-6

The complete mitochondrial genome of *Spilonota lechriaspis* Meyrick (Lepidoptera: Tortricidae)

Jin-Liang Zhao · Yan-Yan Zhang · A-Rong Luo · Guo-Fang Jiang · Stephen L. Cameron · Chao-Dong Zhu

Received: 22 July 2010 / Accepted: 9 November 2010 / Published online: 24 November 2010
© Springer Science+Business Media B.V. 2010

Abstract We determined the nucleotide sequence of the mitochondrial genome (mtgenome) of *Spilonota lechriaspis* Meyrick (Lepidoptera: Tortricidae). The entire closed circular molecule is 15,368 bp and contains 37 genes with the typical gene complement and order for lepidopteran mtgenomes. All tRNAs except $tRNA^{Ser(AGN)}$ can be folded into the typical cloverleaf secondary structures. The protein-coding genes (PCGs) have typical mitochondrial start codons, with the exception of COI, which uses the unusual CGA one as is found in all other Lepidoptera sequenced to date. In addition, six of 13 PCGs harbor the incomplete termination codons, a single T. The A + T-rich region contains some conserved structures that are similar to those found in other lepidopteran mtgenomes, including a structure combining the motif 'ATAGA', a 19-bp poly(T) stretch and three microsatellite $(AT)_n$ elements which are part of larger 122+ bp macrorepeats. This is the first report of macrorepeats in a lepidopteran mtgenome.

Keywords Mitochondrial genome · Lepidoptera · Tortricidae · *Spilonota lechriaspis* Meyrick

J.-L. Zhao · Y.-Y. Zhang · G.-F. Jiang (✉)
Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing 210046, China
e-mail: cnjgf1208@163.com

J.-L. Zhao · A.-R. Luo · C.-D. Zhu (✉)
Key Laboratory of Zoological Systematics and Evolution (CAS), Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
e-mail: zhucd@ioz.ac.cn

S. L. Cameron
Australian National Insect Collection, CSIRO Entomology, P. O. Box 1700, Canberra, ACT 2601, Australia

Introduction

Complete mitochondrial genome (mtgenome) sequences are seeing wider use as molecular markers for phylogenetic, phylogeographic and ecological studies of insects [1–5] plus as a model system for genome biology [6–9]. Wolstenholme and Clary [10] reported the first insect mtgenome sequence in 1985 for *Drosophila*. Up to 2006, there had been 46 complete or near complete mtgenome sequences lodged in GenBank [11], and the number has rapidly increased in the last five years, with to 198 ones in April, 2010. The mtgenome of insects as with other metazoans is a closed-circular molecule ranging in size from 14 to 20 kb, which encodes 37 genes, consisting of 13 PCGs, two ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes [12]. Additionally, it contains a major non-coding area, termed the control region or the A + T-rich region, which regulates and initiates replication and transcription [13].

The major insect order Lepidoptera, the moths and butterflies, has more than 160,000 described species and a cosmopolitan distribution [14]. The family Tortricidae is one of the largest lepidopteran families with more than 5,000 described species and several serious pests of the forests, agricultural crops and fruit trees [15]. The larvae eat leaves, stems and roots, and folivorous species include leaf rollers, leaf webbers, and leaf miners [16]. Species in the genus *Spilonota* have a Holarctic distribution including China, Japan, Korea, Russia, North America and European regions [17]. *Spilonota* includes notorious pest species of fruit and tea trees with the larvae feeding primarily on leaves as leaf rollers, but also occasionally attacking fruits [18].

The identification of species in the genus *Spilonota* is difficult and complicated, because of the similarity of

morphological characteristics and genital structures across the genus. So it is not straightforward to definitively identify them, and some species of the genus are vaguely delimited till now. Despite a lot of studies have been reported about the morphological, physiological and biological features, few have been done on molecular biology yet. And the ranges of the host species are apple, pear, loquat, hawkthorn and so on [17]. It is a common pest of apple and pear. So we get more studied on molecular biology. The present study describes the complete mtgenome of *Spilonota lechriaspis*, a second mtgenome from the family of Tortricidae, and the first one for the subfamily of Olethreutinae.

Materials and methods

Specimen collection and DNA extraction

Individual larvae of *S. lechriaspis* were collected in an organic apple orchard in Beijing, China. Raised in the laboratory, the hatched adults were collected, preserved in 100% ethanol and stored at −20°C. DNA extraction from a single specimen was performed using the DNeasy Tissue kit (QIAGEN) following the manufacturer instructions.

Primers design and PCR amplification

First, short fragment amplifications were performed with the universal PCR primers from Simon et al. [19] and tortricid specific primers from Lee et al. [11] (Table 1). Primer pairs to amplify the Gln-ND2 and whole COI were designed by comparison of lepidopteran sequences available in the GenBank. Species-specific primers were designed using Primer Premier 5.0 software and sequence alignments of the complete mtgenome of *Adoxophyes honmai* [11]. PCR products covering the remaining regions of the mtgenome were amplified using universal and species-specific primers (see Table 1). The entire genome of *S. lechriaspis* was amplified in 15 fragments (Table 1). All the primers were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Beijing, China). In order to decrease time in sequencing and walking, we used the standard PCR technique rather than long PCR techniques. PCR conditions were as follows: an initial denaturation for 5 min at 95°C, followed by 34 cycles of denaturation 30 s at 94°C, annealing 30 s at 50–55°C (depending on primer combinations), elongation 1–3 min (depending on putative length of the fragments) at 68°C, a final extension step of 72°C for 10 min. For most fragments, we used 2× Taq PCR MasterMix (TIANGEN BIOTECH CO., LTD., Beijing, China) in the amplification, but the srRNA-ND2 region was amplified using Takara LA Taq (Takara Co., Dalian, China). All amplifications were performed on an Eppendorf Mastercycler and Mastercycler gradient in 50 μl reaction volumes. The reaction volume of 2× Taq PCR MasterMix contains of 22 μl sterilized distilled water, 25 μl 2× Master Mix, 1 μl of each primer (10 μM) and 1 μl of DNA template; and the one of Takara LA Taq consists of 26.5 μl of sterilized distilled water, 5 μl of 10× LA PCR Buffer II (Takara), 5 μl of 25 mM $MgCl_2$, 8 μl of dNTPs Mixture, 2 μl of each primer (10 μM), 1 μl of DNA template and 0.5 μl (1.25 U) of TaKaRa LA Taq polymerase (Takara).

Cloning, sequencing and sequence assembling

The PCR products were detected via electrophoresis in 1% agarose gel, purified using the 3S Spin PCR Product Purification Kit, and sequenced directly with ABI-377 automatic DNA sequencer. All amplified products were sequenced directly using upstream and downstream primers from bi-directions except the COIII-ND5, ND5-ND4 and srRNA-ND2, which were sequenced after cloning. They were ligated to the *pEASY*-T3 Cloning Vector (Beijing TransGen Biotech Co., Ltd., Beijing, China), and then sequenced by M13-F and M13-R primers and walking. Sequencing was performed using ABI BigDye ver 3.1 dye terminator sequencing technology and run on ABI PRISM 3730 × 1 capillary sequencers. Raw sequences were manually checked and assembled using the software BioEdit [20], Chromas 2.22 and SeqMan (DNAStar, Steve, 2001).

Gene annotation and analysis

Transfer RNA genes analysis was found using tRNAscan-SE software v.1.21 [21]. Putative tRNAs, which were not found by tRNAscan-SE were confirmed by sequence alignment with the homologous gene in *A. honmai*. Secondary structure was manually inferred using DNASIS (Ver.2.5). Annotation was checked by comparison with tRNA determined for other Lepidoptera. Protein-coding genes (PCGs) were identified by similarity of inferred open reading frames to published lepidopteran mt gene amino acid sequences. Translation of PCG open reading frames was made in MEGA ver4.0 [22]. Ribosomal RNA genes (rRNAs) were identified by NCBI Internet BLAST search and annotated with reference to secondary structure features.

Results and discussion

Genome organization and base composition

The mtgenome of *S. lechriaspis* is a closed-circular molecule of 15,368 bp and has been lodged with GenBank

Table 1 Region, primers and sequences for PCRs in this study

Region	Primer pairs (F/R)	Sequennce (forward and reverse) 5′ → 3′
Gln-ND2	Gln10486[a]/ND2-N-784[b]	TAAACTATATCTAATAATATCAAAAATTATTGTGC/TTTAATCCTCCGATAGCTCCAAT
ND2-COI	ND2-J-687[b]/COI-N-1636[b]	ATTTCCCCTATTATTCTTCTTTC/CAATAGTATTATAAATTTGATCATC
COI	COIF1[b]/COIR1[b]	TATCGCYTAWAHCTCAGCCA/TCAWGTTGCCATTTCTA
Leu-Lys	Leu-J-3029[b]/Lys14111Re[a]	CTAATATGGCAGACTATATGTAATGGA/GACCATTACTTGCTTTCAGTCATCTAATG
COII-COIII	C2-J-3696[d]/C3-N-4732[b]	GAAATTTGCGGAGCAAACCATAG/ACTTCACTAGCATAAAGATTTC
Lys-COIII	Lys14111[a]/C3-N-5460[d]	CATTAGATGACTGAAAGCAAGTAATGGTC/TCAACAAAGTGTCAATATCA
COIII-ND5	C3-J-5098[b]/ND5-2183Re[a]	TTGAAATTGGGATAATATGACCT/TGCTGGCATATTAACTGCTTTATCT
ND5-ND4	ND5-2183[a]/N4-N-8718[d]	AGATAAAGCAGTTAATATGCCAGCA/GCCTATTCATCWGTTGCTCA
ND4-CytB	N4-J-8479[b]/CB-J-11139[b]	ATATTCCCGTGAAACTCCTATAC/ATTACAGTTGCTCCTCAGAATGA
CytB	CB-J-10612[d]/CB-N-11367[d]	CATCTAACATCTCAGCATGATGAA/TAACTCCTCCTAATTTATTGGGA
CytB-lrRNA	CytB-5971[a]/LR-N-12866[c]	CAAACAGGATCTAATAACCCTTTAGG/ACATGATCTGAGTTCAAACCGG
ND1-lrRNA	N1-J-12585[c]/LR-N-13398[c]	GGTCCCTTACGAATTTGAATATATCCT/CGCCTGTTTAACAAAAACAT
lrRNA-srRNA	LR-J-13375[d]/SR-N-14588[c]	TCAGTGGGCAGATTAGAC/AAACTAGGATTAGATACCCTATTAT
srRNA	SR-J-14233[d]/SR-N-14588[c]	GAAAGCGACGGGCAATATG/AAACTAGGATTAGATACCCTATTAT
srRNA-ND2	12S-9493[a]/N2-N-199[b]	TTTTAATAATAGGGTATCTAATCCTAGTTTTT/CAATTCAACATCCAAATCAAGAA

[a] Primers from Lee et al. [11]

[b] Primers newly designed for this genome

[c] Primers from Simon et al. [19]

[d] Primers modified from Simon et al. [19] up to this mtgenome

(sequence accession number HM204705). It encodes 13 PCGs, 22 tRNAs and 2 rRNAs. The arrangement of mitochondrial genes is the same as that reported for other Lepidoptera, and differs from the ancestral insect mtgenome by the rearrangement of tRNA^{Met}. This mtgenome includes 18 non-coding regions, ranging in size from 1 to 441 bp, among which seven spacers span longer than 10 bp; a total of 21 bp overlapping nucleotides scatter in 5 locations. The longest overlapping nucleotides fragment is 8 bp long, located between tRNA^{Trp} and tRNA^{Cys}, which overlap in many insect species.

Composition of the J-strand of mtDNA is 39.9% A, 41.3% T, 7.6% G and 11.2% C, with a total A + T content of 81.2%. This value is well in the range of the other arthropods, which show a remarkable variability, from 69.5 to 84.9% [23, 24], and is slightly higher than that of *A. honmai* (80.4%).

Protein-coding genes and codon usage

The mtgenome of *S. lechriaspis* contains the canonical 13 PCGs present in most bilateral animals. The initial and terminal codons of all PCGs are shown in Table 2. 12 PCGs utilize the standard ATN start codons (ATT for ND2, ATP8, ND3, ND5, ND6; ATG for COII, ATP6, COIII, ND4, ND4L, CytB, ND1). COI in *S. lechriaspis* has the start codon CGA (Arg) as in *A. honmai* and most other lepidopteran species sequenced to date [25]. It is noteworthy that the COI gene often starts with non-standard putative codons, which have been extensively discussed about for several arthropod species [26]. Alternatively, there is also 4-bp stretch, TTAG within tRNA^{Tyr}, which has been proposed as a non-standard start codon for the COI gene in other insects. This stretch is found in several lepidopteran species, such as *Bombyx* [27, 28], *Coreana raphaelis* [29], *Antheraea pernyi* [30], and *Antheraea yamamai* [31] (Fig. 1); however it is not universal in Lepidoptera whereas the CGA codon is found in all species studied to date. More studies for mRNA transcripts are needed to clarify the prediction of COI initiation; however, one of the few mRNA studies of this region in Lepidoptera doesn't support the utilization of tetrad start codons in this order [32].

Six PCGs (ND2, COI, COII, ND5, ND4 and CytB) have incomplete stop codons consisting of just a T-nucleotide, and others stop with standard terminal codon (TAA). There is also a high degree of conservation of incomplete stop codon across the order. COI and COII usually have incomplete stop codon in lepidopteran species, such as *C. raphaelis* [29], *A. honmai* [11], *Bombyx mandarina* [28], *Phthonandria atrilineata* [33], *Manduca sexta* [34], *Ochrogaster lunifer* [35], *A. pernyi* [30], *Artogeia melete* [36], *Eriogyna pyretorum* [37]. The common interpretation of this phenomenon is that TAA terminator is created via post-transcriptional polyadenylation [38].

Table 2 Organization of the *S. lechriaspis* Meyrick mitochondrial genome

Gene	Direction	Location	Size (bp)	IN	Anticodon	Start codon	Stop codon
$tRNA^{Met}$	F	1–67	67		CAU		
$tRNA^{Ile}$	F	70–134	65	2	GAU		
$tRNA^{Gln}$	R	132–200	69	−3	UUG		
ND2	F	248–1256	1009	47		ATT	T
$tRNA^{Trp}$	F	1257–1324	68	0	UCA		
$tRNA^{Cys}$	R	1317–1381	65	−8	GCA		
$tRNA^{Tyr}$	R	1403–1467	65	21	GUA		
COI	F	1471–3001	1531	3		CGA	T
$tRNA^{Leu(UUR)}$	F	3002–3068	67	0	UUA		
COII	F	3069–3750	682	0		ATG	T
$tRNA^{Lys}$	F	3751–3819	69	0	CUU		
$tRNA^{Asp}$	F	3822–3887	66	2	GUC		
ATP8	F	3888–4049	162	0		ATT	TAA
ATP6	F	4043–4720	678	−7		ATG	TAA
COIII	F	4730–5518	789	9		ATG	TAA
$tRNA^{Gly}$	F	5521–5586	66	2	UCC		
ND3	F	5587–5940	354	0		ATT	TAA
$tRNA^{Ala}$	F	5952–6017	66	11	UGC		
$tRNA^{Arg}$	F	6030–6094	65	12	UCG		
$tRNA^{Asn}$	F	6096–6160	65	1	GUU		
$tRNA^{Ser(AGN)}$	F	6164–6229	66	3	GCU		
$tRNA^{Glu}$	F	6230–6295	66	0	UUC		
$tRNA^{Phe}$	R	6294–6358	65	−2	GAA		
ND5	R	6359–8075	1717	0		ATT	T
$tRNA^{His}$	R	8091–8156	66	15	GUG		
ND4	R	8157–9495	1339	0		ATG	T
ND4L	R	9495–9785	291	−1		ATG	TAA
$tRNA^{Thr}$	F	9794–9857	64	8	UGU		
$tRNA^{Pro}$	R	9858–9923	66	0	UGG		
ND6	F	9926–10453	528	2		ATT	TAA
CytB	F	10461–11607	1147	7		ATG	T
$tRNA^{Ser(UCN)}$	F	11608–11674	67	0	UGA		
ND1	R	11693–12631	939	18		ATG	TAA
$tRNA^{Leu(CUN)}$	R	12633–12700	68	1	UAG		
lrRNA	R	12701–14083	1383	0			
$tRNA^{Val}$	R	14084–14149	66	0	UAC		
srRNA	R	14150–14927	778	0			
A + T		14928–15368	441				

IN intergenic nucleotides

Fig. 1 The start codon of COI in some species

```
Antheraea pernyi        - - - - - - - - A A T T A G C G A A A A T G A C T T T A - - - - - -
Antheraea yamamai       - - - - - - - - . . . . . . . . . . . . . . . . . . . . - - - - - - -
Bombyx mandarina        T T A C T T T T . T . . . . . . . . . . . . . . A . . . - - - - - - -
Bombyx mori             T T T T T C T T T T . . . . . . . . . . . . . . A . . . T T C T A C
Coreana raphaelis       - - - - - - - - - - . . . . . . . . . . . . . . . . . . T T T C - - -
Spilonota lechriaspis   - - - - T T T T . T . . . . . . . . . . . . . . A . C - - - - - - - -
```

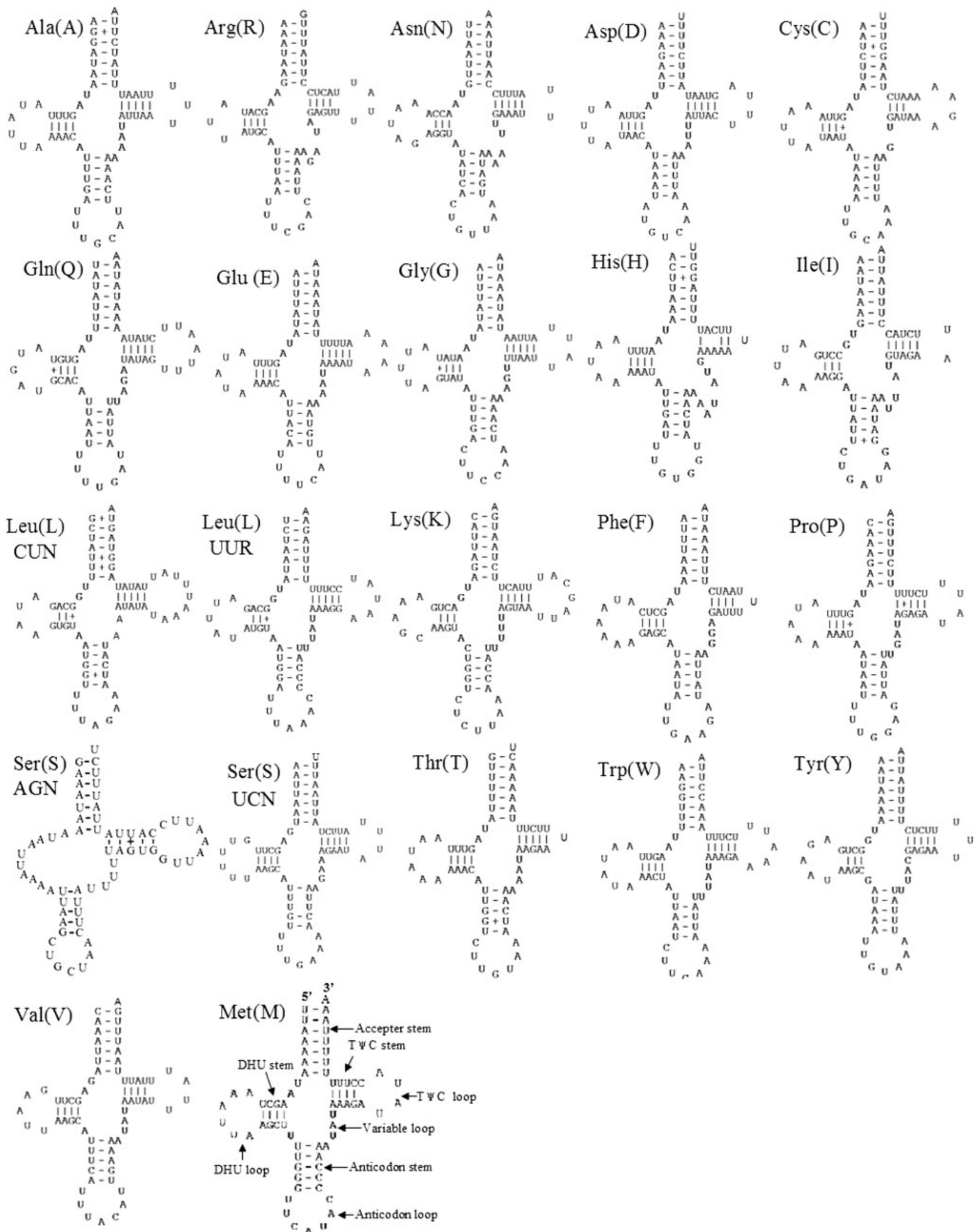

Fig. 2 Putative secondary structures for the tRNA genes of the *S. lechriaspis* mtgenome

Transfer RNA and ribosomal RNA genes

The *S. lechriaspis* mtgenome contains the typical set of 22 tRNA genes, which are intersperse between rRNAs and PCGs. Fourteen tRNAs are coded on the J-strand and eight on the N-strand, which is the same organization as in other lepidopterans. Complete cloverleaf secondary structures can be inferred for 21 of the 22 tRNAs, with $tRNASer^{(AGN)}$ lacking the DHU arm (Fig. 2). Anticodon sequences for *S. lechriaspis* tRNAs are all identical to those found in homologous tRNA isotypes across Lepidoptera. 19 tRNA genes show 40 pairs of mismatches in their stems, including 12 pairs in the DHU stems, nine pairs in the amino acid acceptor stems, nine pairs in the TΨC stems and ten pairs in the anticodon stems. The mismatched bases are mainly G.U, U.G, U.U, AC or AA, and some are A.G, G.A, G.G or C.G ones. These mismatches are possibly corrected by RNA-editing mechanisms [39].

As in other mtgenome sequences, there are two rRNA genes in *S. lechriaspis,* small and large ribosomal genes (srRNA and lrRNA). The lrRNA locates between $tRNA^{Leu(CUN)}$ and $tRNA^{Val}$, and srRNA between $tRNA^{Val}$ and the A + T-rich region. The length of lrRNA of *S. lechriaspis* is 1,382 bp long with 85.17% A + T content; and the srRNA is 778 bp with 86.25% A + T. The length is well located in the range 1,330 bp (*C. raphaelis* [29]) to 1,412 bp (*Diatraea saccharalis* [Unpublished, GenBank accession number NC_013274]), 806 bp (*O. lunifer* [35]) to 774 bp (*Caligula boisduvalii* [40]), respectively. There is two (TA)n microsatellites in the range of lrRNA, one is $(TA)_{13}$ and the other is $(TA)_{12}$. The *A. honmai* has only one (i.e., $(TA)_{16}$) at the location of the first one, and a little larger than *S. lechriaspis*.

Non-coding and overlapping regions

In addition to the A + T-rich region (the largest non-coding region), the mtgenome of *S. lechriaspis* contains 17 non-coding regions, ranging from 1 to 47 bp and dispersing throughout the whole genome (Table 1). The length of the 17 non-coding regions is 164 bp in total and content of A + T is much more than that of G + C (i.e., 92.1 vs. 7.9%) (Table 1). The larger non-coding region is 47 bp long between $tRNA^{Gln}$ and ND2, with the A + T content of 89.4%, which is lower for this non-coding region than in the equivalent region found in other lepidopterans, which typically has a higher A + T content (93-100%) has been reported [27, 41]. Besides this non-coding region, there is another more interesting one between $tRNA^{Ser(UCN)}$ and ND1. This region is common to most insect mtgenomes, such as Lepidoptera, Coleoptera and Hymenoptera, and may correspond to the binding site of mtTERM, a transcription attenuation factor [13]. In Lepidoptera, all the species in the GenBank up to date have a 7 bp motif (ATACTAA) [34] including *S. lechriaspis* in this paper. In Coleoptera, there is a 5 bp motif (TACTA) [42]. And in Hymenoptera, the motif is a little lesser extended, with a 6-bp conserved motif (THACWW) [43]. Obviously this motif of Lepidoptera is the most conserved one. And there are five overlapping regions varying in size from 1 to 8 bp (21 bp total).

A + T-rich region

The A + T-rich region of *S. lechriaspis* is 441 bp with 92.74% A + T content, and 48 bp longer than the most closely related species for which mtgenome data is available (*A. honmai*). It regulates the transcription and replication of the mtgenome [44], and includes the O_N (origin of minority or light strand replication) identified by the motif ATAGA, which is located at 16 bp upstream from srRNA and followed by a 19 bp long polyT stretch. This O_N motif is conserved among all lepidopterans; however, the polyT stretch varies in length between species ranging in size from 18 to 22 bp. We have an important finding that there are three tandem 122 + bp repeats (plus one partial repeat 27 bp in length). The tandem repeats vary only in the size of an $(AT)_n$ microsatellite, which is located roughly in the middle of the repeat. Due to variation in the number of AT repeats, the tandem repeats are 128, 124 and 122 bp long, with the repeats containing 10, 8 and 7 dinucleotide repeats respectively (Fig. 3). This is the first report of AT region macrorepeats within lepidopterans, but they are common in other insects where they have been reported to include

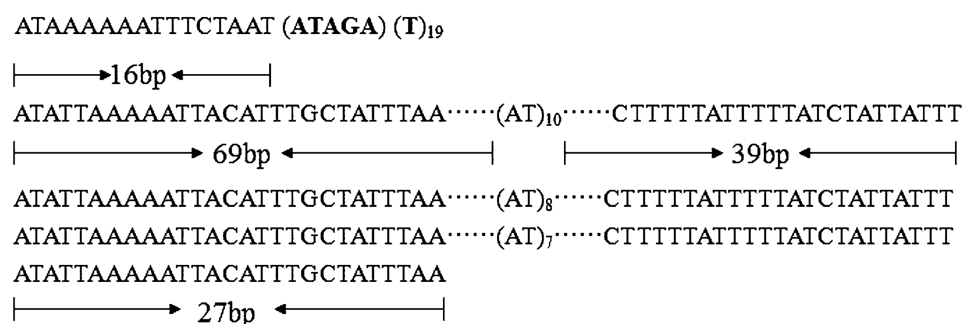

Fig. 3 The similar tandem repeats of *S. lechriaspis* in A + T rich region

compound macrorepeats in termites [45] or even tRNA tandem repeats in wasps [46].

Acknowledgments Prof. Qi-Lian Qin and his lab members (Institute of Zoology, Chinese Academy of Sciences) kindly provide advices and facilities in sequence cloning. We also thank Wen-juan Xuan and Na-na Liu (College of Life Sciences, Nanjing Normal University), Fu-qiang Chen, Feng Yuan, Wen-fu Li (Institute of Zoology, Chinese Academy of Sciences) for their comments on the manuscript and kindly help in specimen identification or data analysis. This project was supported mainly by Beijing Municipal Natural Science Foundation (6081002), the Public Welfare Project from the Ministry of Agriculture, China (Grant no. 200803006), and partially by the Innovation Program in the Chinese Academy of Sciences (KSCX2-YW-NF-02), grants from the National Science Foundation, China (30870268, J0930004) to Chaodong Zhu.

References

1. Fenn JD, Song H, Cameron SL, Whiting MF (2008) A mitochondrial genome phylogeny of Orthoptera (Insecta) and approaches to maximizing phylogenetic signal found within mitochondrial genome data. Mol Phylogenet Evol 49:59–68
2. Dowton M, Cameron SL, Austin AD, Whiting MF (2009) Phylogenetic approaches for the analysis of mitochondrial genome sequence data in the Hymenoptera—a lineage with both rapidly and slowly evolving mitochondrial genomes. Mol Phylogenet Evol 52:512–519
3. Cameron SL, Sullivan J, Song H, Miller KB, Whiting MF (2009) A mitochondrial genome phylogeny of the Neuropterida (lacewings, alderflies and snakeflies) and their relationship to the other holometabolous insect orders. Zool Scr 38:575–590
4. Sheffield NC, Song H, Cameron SL, Whiting MF (2009) Nonstationary evolution and compositional heterogeneity in beetle mitochondrial phylogenomics. Syst Biol 58:381–394
5. Song H, Sheffield NC, Cameron SL, Miller KB, Whiting MF (2010) What happens when the phylogenetic assumptions are violated?: the effect of base compositional heterogeneity and among-site rate heterogeneity in beetle mitochondrial phylogenomics. Syst Entomol 35:429–448
6. Covacin C, Shao R, Cameron SL, Barker SC (2006) Extraordinary amounts of gene rearrangement in the mitochondrial genomes of lice (Insecta: Phthiraptera). Insect Mol Biol 15:63–68
7. 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
8. Sheffield NC, Song H, Cameron SL, Whiting MF (2008) A comparative analysis of mitochondrial genomes in Coleoptera (Arthropoda: Insecta) and genome descriptions of six new beetles. Mol Biol Evol 25:2499–2509
9. Dowton M, Cameron SL, Dowavic JI, Austin AD, Whiting MF (2009) Characterization of 66 mitochondrial gene rearrangements in the Hymenoptera reveal underlying trends in mitochondrial genome evolution. Mol Biol Evol 26:1607–1617
10. Wolstenholme DR, Clary DO (1985) Sequence evolution of *Drosophila* mitochondrial DNA. Genetics 109:725–744
11. Lee ES, Shin KS, Kim MS, Park H, Cho S, Kim CB (2006) The mitochondrial genome of the smaller tea tortrix *Adoxophyes honmai* (Lepidoptera: Tortricidae). Gene 373:52–57
12. Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Res 27:1767–1780
13. Taanman JW (1999) The mitochondrial genome: structure, transcription, translation and replication. Biochim Biophys Acta 1410:103–123
14. Hu J, Zhang DX, Hao JS, Huang DY, Cameron S, Zhu CD (2009) The complete mitochondrial genome of the yellow coaster, Acraea issoria (Lepidoptera: Nymphalidae: Heliconiinae: Acraeini): sequence, gene organization and a unique tRNA translocation event. Mol Biol Rep. doi:10.1007/s11033-009-9934-3
15. Horak M, Brown RL (1991) Tortricid pests, their biology, natural enemies and control. Elsevier, Amsterdam, pp 23–48
16. Roelofs WL, Brown RL (1982) Pheromones and evolutionary relationships of Tortricidae. Annu Rev Ecol Syst 13:396–422
17. Liu YQ, Li GW (2002) Fauna sinica, insect vol.27, Lepidoptera,Tortricidae. Science Press, Beijing, pp 315–316
18. Park KT, Cheo KY, Paik JC, Han SC (1977) Lepidopterous insect pest on apple tree. Korean J Plant Prot 16(1):33–39
19. Simon C, Frati F, Bekenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain-reaction primers. Ann Entomol Soc Am 87:651–701
20. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
21. 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
22. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
23. Crozier RH, Crozier YC (1993) The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. Genetics 133:97–117
24. Dotson EM, Beard CB (2001) Sequence and organization of the mitochondrial genome of the Chagas disease vector, *Triatoma dimidiata*. Insect Mol Biol 10(3):205–215
25. 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
26. Caterino MS, Sperling FA (1999) *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. Mol Phylogenet Evol 11:122–137
27. 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 silkmoth, *Bombyx mandarina*, and its close relative, the domesticated silkmoth, *Bombyx mori*. Mol Biol Evol 19: 1385–1389
28. Pan MH, Yu QY, Xia YL, Dai FY, Liu YQ, Lu C, Zhang Z, Xiang ZH (2008) Characterization of mitochondrial genome of Chinese wild mulberry silkworm, *Bombyx mandarina* (Lepidoptera: Bombycidae). Sci China Ser C 51(8):693–701
29. 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
30. Liu YQ, Li YP, Pan MH, Dai FY, Zhu XW, Lu C, Xiang ZH (2008) The complete mitochondrial genome of the Chinese oak silkmoth, *Antheraea pernyi* (Lepidoptera: Saturniidae). Acta Biochim Biophys Sin (Shanghai) 40(8):693–703
31. Kim SR, Kim MI, Hong MY, Kim KY, Kang PD, Hwang JS, Han YS, Jin BR, Kim I (2009) The complete mitogenome sequence of the Japanese oak silkmoth, *Antheraea yamamai* (Lepidoptera: Saturniidae). Mol Biol Rep 36(7):1871–1880
32. Frohlich DR, Stevenson BA, Peterson AM, Wells MA (1996) Mitochondrial cytochrome C oxidase subunit I of *Manduca sexta*

and a comparison with other invertebrate genes. Comp Biochem Physiol B 113B:785–788

33. Yang L, Wei ZJ, Hong GY, Jiang ST, Wen LP (2009) The complete nucleotide sequence of the mitochondrial genome of *Phthonandria atrilineata* (Lepidoptera: Geometridae). Mol Biol Rep 36:1441–1449
34. Cameron SL, 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
35. Salvato P, Simonato M, Battisti A, Negrisolo E (2008) The complete mitochondrial genome of the bag-shelter moth *Ochrogaster lunifer* (Lepidoptera, Notodontidae). BMC Genomics 9:331
36. Hong G, Jiang ST, Yu M, Yang Y, Li F, Xue F, Wei ZJ (2009) The complete nucleotide sequence of the mitochondrial genome of the cabbage butterfly, *Artogeia melete* (Lepidoptera: Pieridae). Acta Biochim Biophys Sin 41(6):446–455
37. Jiang ST, Hong GY, Yu M, Li N, Yang Y, Liu YQ, Wei ZJ (2009) Characterization of the complete mitochondrial genome of the giant silkworm moth, *Eriogyna pyretorum* (Lepidoptera: Saturniidae). Int J Biol Sci 5(4):351–365
38. Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature 290:470–474
39. 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
40. Hong MY, Lee EM, Jo YH, Park HC, Kim SR, Hwang JS, Jin BR, Kang PD, Kim KG, 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(1–2): 49–57
41. Coates BS, Sumerford DV, Hellmich RL, Lewis LC (2005) Partial mitochondrial genome sequence of *Ostrinia nubilalis* and *Ostrinia furnicalis*. Int J Biol Sci 1:13–18
42. Sheffield NC, Song H, Cameron SL, Whiting MF (2008) A comparative analysis of mitochondrial genomes in Coleoptera (Arthropoda: Insecta) and genome descriptions of six new beetles. Mol Biol Evol 25(11):2499–2509
43. Wei SJ, Tang P, Zheng LH, Shi M, Chen XX (2010) The complete mitochondrial genome of Evania appendigaster (Hymenoptera: Evaniidae) has low A + T content and a long intergenic spacer between atp8 and atp6. Mol Biol Rep 37:1931–1942
44. Clayton DA (1992) Transcription and replication of animal mitochondrial DNA. Int Rev Cyt 141:217–232
45. Cameron SL, Whiting MF (2007) Mitochondrial genomic comparisons of the subterranean termites from the Genus *Reticulitermes* (Insecta: Isoptera: Rhinotermitidae). Genome 50:188–202
46. 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