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Mitochondrial Genomic Analysis of the Tea Geometrid, *Ectropis grisescens* Warren, 1894 (Lepidoptera: Geometridae) and Its Phylogenetic Implications

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ABSTRACT

Ectropis grisescens Warren, 1894 is a major pest species only feeding on tea plants but lacks sufficient molecular data to clarify its genetic characters. Complete mitochondrial genome of the tea geometrid *Ectropis grisescens* is sequenced for the first time. The mitogenome of *E. grisescens* is 16,575 bp in length, with an A+T content of 81.2%. The typical set of 37 genes (13 PCGs, 22 tRNA genes and two rRNA genes) are all identified. Most PCGs have standard ATN start codons and TAN stop codons. Most tRNA genes exhibite cloverleaf secondary structures, whereas the dihydrouridine (DHU) arm of *trnSer (AGN)* is reduced into a small loop. Poly-T stretch, tandem repeats and stem-loop (SL) structures are predicted in the control region, which is long (1550 bp) and highly biased toward A+T nucleotides (92.3%). The mitochondrial phylogenetic analyses using the Bayesian inference (BI) and maximum likelihood methods (ML) consistently recover the generic relationships within Geometridae. *E. grisescens* is correctly grouped with its congener and the monophyly of Ennominae and Larentiinae are both supported.

Key words: Lepidoptera, Geometridae, *Ectropis grisescens*, Mitochondrial genome.

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INTRODUCTION

Mitochondrial genome (mitogenome) has become one of the most popular molecules used in recent phylogenetic studies. The advantages of using mitogenomic data in phylogenetic studies include low cost, sufficient genetic information for higher taxa, and low rate of rearrangement, etc (Boore, 1999). Meanwhile, the mitochondrial cytochrome oxidase subunit I (*cox1*) gene is widely used for DNA barcoding in species delimitation and intraspecific genetics (Hebert, Cywinska, Ball, & deWaard, 2003). Structures of animal mitogenomes are usually conserved with 37 typical set of genes, including13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes and two ribosomal RNA (rRNA) genes (Simon, Buckley, Frati, Stewart, & Beckenbach, 2006).

Lepidoptera is the second largest insect order with about 15,7000 species, and many of them are major economic pests (Stork, 2018; Mullen & Zaspel, 2019). Geometridae is one of the largest families in Lepidoptera, comprising over 21,000 species usually harmful to the agriculture and forestry and about 2000 of which are found in China (Scoble, 1999; Gu & Xin, 2018). Among the considerable large number of geometrids, only 12 species have sequenced mitogenomes available from GenBank. The limited mitogenomes are out of proportion to the large number of species in Geometridae.

Ectropis grisescens Warren, 1894 also known as the tea geometrid, is an economically important pest species in Asia and is especially widely distributed in the tropical and subtropical areas of China (Warren, 1894; Jiang et al, 2014). Larvae of *E. grisescens* prefer to damage the bud and leaves of the tea plants, causing considerable economic loss in the tea industry of China (Ge, Yan & Xiao, 2015). Accumulation of genetic data of *E. grisescens* is warranted to find new control strategies for this pest from a molecular view. However, the mitogenome of *E. grisescens* is still undetermined, hindering our understanding of the relationship between *E. grisescens* and other lepidopteran pests. In this study, the complete mitogenome of *E. grisescens* is sequenced and described for the first time. The mitogenomic structure, nucleotide compositions, codon usages of PCGs, secondary structures of tRNA genes and the control region are investigated to better understand the genetic characters of this important pest.

MATERIALS AND METHODS

Sampling and DNA extraction

Adult specimens of *E. grisescens* were collected from Wolong Town, Qionglai City, Sichuan Province of China in May of 2019. The specimens were identified by Sichuan Academy of Agricultural Sciences and preserved in 100% ethanol. Total genomic DNA was extracted using the E.Z.N.A.® Tissue DNA Kit (OMEGA, America) and stored at -20 °C until used for mitogenome sequencing.

Mitogenome sequencing, assembly and annotation

After DNA extraction, 1.0 μg of purified DNA was fragmented and used to construct Illumina TruSeq short-insert libraries (insert size = 450 bp) following the manufacturer's instructions, then sequenced on the Illumina Hiseq 4000 (Shanghai BIOZERON Co., Ltd). Raw reads were filtered prior to assembly. The reads with adaptors, with a low quality score below 20, with over 10% "N" bases, or with very few nucleotides were removed. High-quality reads were used for de novo and reference-guided assembling according to the following steps: 1) Firstly, the filtered reads were assembled into contigs using SOAPdenovo2.04 (Luo et al, 2012); 2) Secondly, the contigs were aligned to the reference mitogenome of *Abraxas suspecta* Warren, 1894 (GenBank accession number KY095828) using BLAST; the aligned contigs (≥80% similarity and query coverage) were ordered according to the reference mitogenome; 3) Finally, the clean reads were mapped to the assembled draft mitogenome to calibrate the wrong bases, and the gaps were filled by GapFiller v2.1.1 (https://sourceforge.net/projects/gapfiller/).

The complete mitogenome sequence of *E. grisescens* was deposited into GenBank under the accession number MN792921. Location and secondary structures of the tRNA genes were predicted by MITOS (Bernt et al, 2013). PCGs and rRNA genes were annotated using homology alignments. Open reading frames of PCGs were examined and adjusted by ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/). CGView Server (http://stothard.afns.ualberta.ca/cgview_server/) was used to illustrate the mitogenome of *E. grisescens* (Grant & Stothard, 2008). Nucleotide composition and codon usage were calculated by MEGA v.6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The two formulas AT-skew = [A–T]/[A+T] and GC-skew = [G–C]/[G+C] were used in the composition skew analysis (Perna & Kocher, 1995). Secondary structures in the control region were predicted by Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.advanced.submit.html) and DNAMAN v6.0.3.

Phylogenetic analyses

PCGs derived from 13 moths of Geometridae, including *E. grisescens* sequenced in this study, were used in the phylogenetic analyses (Table 1). Another moth, *Cameraria ohridella* Deschka & Dimic 1986 from family Gracillariidae (GenBank accession number KJ508042) was used as the outgroup. Each of the 13 PCGs was respectively aligned by MAFFT, then concatenated into a combined dataset using SequenceMatrix v1.7.8 (Katoh & Standley, 2013). PartitionFinder v2.1.1 was used to determine the optimal nucleotide substitution models and partitioning schemes with the Bayesian Information Criterion (BIC) and a greedy search algorithm (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016). Two phylogenetic inferences were conducted, including Bayesian inferences (BI) and Maximum likelihood (ML) analysis. BI analysis was conducted by MrBayes v3.2.6, running 20 million generations, sampling every 1000 generations and executing one cold chain and three hot chains with a burn-in of 25% trees (Ronquist & Huelsenbeck, 2003). All runs of BI analysis were examined by Tracer v.1.5 (Rambaut & Drummond, 2019). ML analysis was performed with RAxML v8, with 1000 bootstrap replicates (Stamatakis, 2014). Both BI and ML trees were depicted with FigTree v1.4.2.

Table 1. Species of Lepidoptera used in the phylogenetic study.

Family	Species	GenBank accession number
Geometridae	*Abraxas suspecta*	KY095828
	Apocheima cinerarium	KF836545
	Biston panterinaria	JX406146
	Biston perclara	KU325536
	Biston suppressaria	KP278206
	Biston thibetaria	KJ670146
	Celenna sp.	KM244697
	Dysstroma truncata	KJ508061
	Ectropis grisescens	KY095828
	Ectropis obliqua	KX827002
	Jankowskia athleta	KR822683
	Operophtera brumata	KP027400
	Phthonandria atrilineata	EU569764
Gracillariidae	*Cameraria ohridella*	KJ508042

RESULTS AND DISCUSSION

Mitogenome structure and nucleotide composition

The complete mitogenome of *E. grisescens* is a double-strand circular molecule with a length of 16,575 bp, longer than all available mitogenomes of Geometridae. The typical set of 37 genes, including 13 PCGs, 22 tRNA genes and two rRNA genes were all identified in the mitogenome. Among these genes, nine PCGs and 14 tRNA genes are encoded by the majority strand (J-strand); the remaining four PCGs, eight tRNA genes and two rRNA genes are on the minority strand (N-strand) (Fig. 1, Table 2). The mitochondrial gene arrangement of *E. grisescens* differs from the putative ancestral mitogenome of *Drosophila yakuba* Burla, 1954 by showing the *trnMet-trnIle-trnGln* tRNA cluster, which is a very common rearrangement pattern in Lepidoptera (Chen & Du, 2016). The mitogenome of *E. grisescens* has 41 overlapping nucleotides in seven gene pairs; the longest overlap is found between *trnPhe* and *nad5*, which is 17-bp long. There are also 238 intergenic nucleotides (IGNs) scattered in 13 gene gaps, indicating a loose mitogenomic structure of *E. grisescens*.

The whole mitogenome of *E. grisescens* is biased toward A+T nucleotides (81.2%) with a positive AT-skew and negative GC-skew as commonly found in most other insects (Table 3) (Wei et al, 2010). The A+T contents are similarly high in the concatenated PCGs, PCGs on J-strand, PCGs on N-strand, tRNA genes and rRNA genes (Table 3). All codon positions of the concatenated PCGs have reversal of strand

asymmetry (negative AT skew and positive GC-skew), whereas tRNA genes and rRNA genes exhibit a positive AT skew and negative GC-skew. In the 37 mitochondrial genes, the A+T content is highest in *trnGlu* (92.3%) and lowest in *cox1* (72.0%).

Table 2. Mitochondrial genome structure of *Ectropis grisescens.*

Gene	Position (bp)	Size (bp)	Direction	Intergenic nucleotides	Anti- or start/stop codons	A+T%
trnMet (M)	1–69	69	Forward	0	CAT	73.9
trnIle (I)	70–137	68	Forward	0	GAT	77.9
trnGln (Q)	135–203	69	Reverse	–3	TTG	84.1
nad2	268–1269	1002	Forward	64	ATA/TAA	84.0
*trnTrp (*W)	1276–1344	69	Forward	6	TCA	78.3
trnCys (C)	1337–1404	68	Reverse	–8	GCA	83.8
trnTyr (Y)	1405–1471	67	Reverse	0	GTA	79.1
cox1	1479–3009	1531	Forward	7	CGA/T	72.0
trnLeu2 (UUR)	3010–3076	67	Forward	0	TAA	73.1
cox2	3077–3758	682	Forward	0	ATG/T	76.8
trnLys (K)	3759–3830	72	Forward	0	CTT	73.6
trnAsp (D)	3833–3898	66	Forward	2	GTC	87.9
atp8	3899–4066	168	Forward	0	ATT/TAA	91.1
atp6	4060–4737	678	Forward	–7	ATG/TAA	79.1
cox3	4741–5529	789	Forward	3	ATG/TAA	72.9
trnGly (G)	5532–5597	66	Forward	2	TCC	90.9
nad3	5595–5951	357	Forward	–3	ATA/TAA	82.9
trnAla (A)	5955–6018	64	Forward	3	TGC	82.8
trnArg (R)	6019–6082	64	Forward	0	TCG	76.6
trnAsn (N)	6083–6147	65	Forward	0	GTT	78.5
trnSer1 (AGN)	6148–6213	66	Forward	0	GCT	80.3
trnGlu (E)	6282–6346	65	Forward	68	TTC	92.3
trnPhe (F)	6349–6414	66	Reverse	2	GAA	83.3
nad5	6398–8152	1755	Reverse	–17	ATC/TAA	81.3
trnHis (H)	8153–8220	68	Reverse	0	GTG	89.7
nad4	8221–9559	1339	Reverse	0	ATG/T	80.5
nad4l	9559–9849	291	Reverse	–1	ATG/TAA	85.6
trnThr (T)	9856–9920	65	Forward	6	TGT	80.0
trnPro (P)	9921–9986	66	Reverse	0	TGG	83.3

Table 2. Continued.

Gene	Position (bp)	Size (bp)	Direction	Intergenic nucleotides	Anti- or start/stop codons	A+T%
nad6	9989–10522	534	Forward	2	ATA/TAA	85.6
cytb	10,564–11,715	1152	Forward	41	ATG/TAA	75.6
trnSer2 (UCN)	11,714–11,779	66	Forward	–2	TGA	81.8
nad1	11,812–12,750	939	Reverse	32	TTG/TAA	77.2
trnLeu1 (CUN)	12,751–12,819	69	Reverse	0	TAG	82.6
rrnL	12,820–14,194	1375	Reverse	0	–	84.5
trnVal (V)	14,195–14,260	66	Reverse	0	TAC	83.3
rrnS	14,261–15,025	765	Reverse	0	–	85.4
Control region	15,026–16,575	1550	–	0	–	91.9

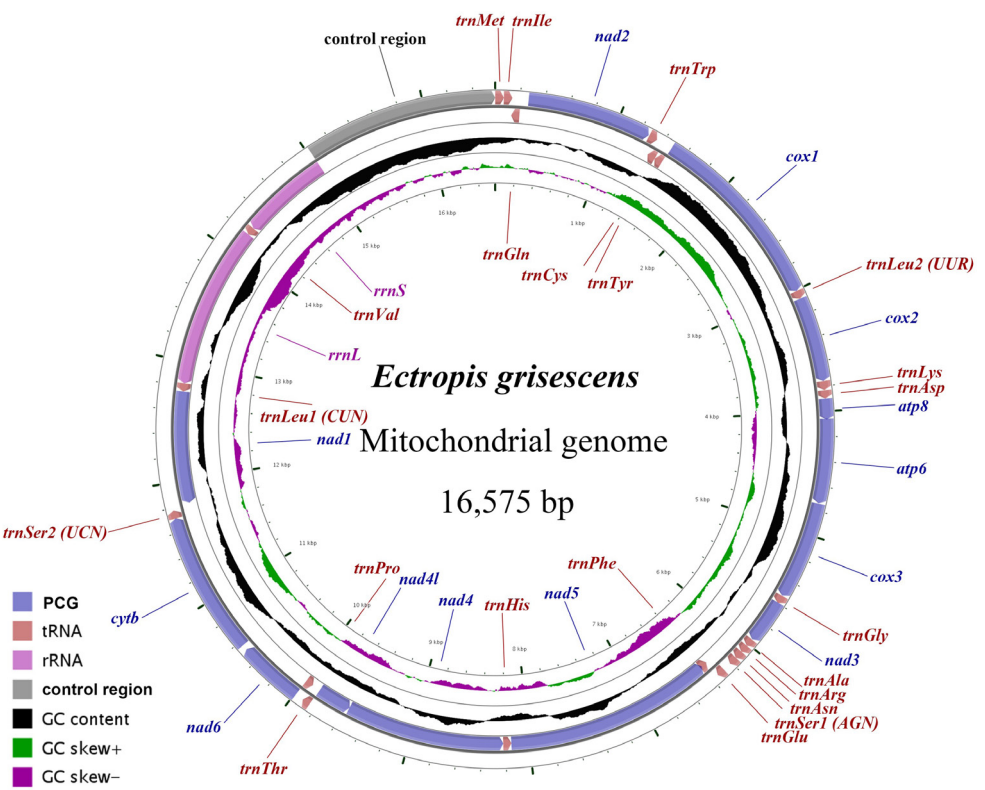

Fig. 1. Mitochondrial map of *Ectropis grisescens*. Genes outside the map are transcribed clockwise; genes inside the map are transcribed counterclockwise. GC content and GC skew are shown as inside circles. GC content and GC skew are plotted as the deviation from the average value of the entire mitogenome sequence.

PCGs, tRNA and rRNA genes

No rearrangement or variations were found in the 13 PCGs of *E. grisescens*. Most PCGs start with the standard ATN codon, including ATA start codon in *nad2*, *nad3* and *nad6*, ATG in *cox2*, *cox3*, *cytb*, *atp6*, *nad4* and *nad4l*, ATT in *atp8* and ATC in *nad5* (Table 2). However, *cox1* uses the special start codon CGA, and *nad1* uses another special start codon TTG. Ten of the PCGs have the complete stop codon TAA, whereas *cox1*, *cox2* and *nad4* contain an incomplete stop codon T, which is common in other lepidopteran species (Garey & Wolstenholme, 1989). The relative synonymous codon usage (RSCU) of the 13 PCGs indicate that TTA (Leu), TCT (Ser) and CGA (Arg) are the most frequently used codons whereas CTG (Leu), CCG (Pro) and AGG (Ser) are the least (Fig. 2).

The regular set of 22 tRNA genes are all found in the mitogenome, while the ancestral *trnIle-trnGln-trnMet* tRNA cluster is rearranged into *trnMet-trnIle-trnGln*. The 22 tRNA genes have a total length of 1471 bp and an A+T content of 81.6%; each tRNA gene ranges in size from 64 to 72 bp (Table 2). Twenty-one of the tRNA genes can fold into typical cloverleaf secondary structures (Fig. 3), however, the TΨC arms of *trnPhe* and *trnThr* are somewhat reduced, and the dihydrouridine (DHU) arm is reduced into a small loop, which is very common in other metazoans (Garey & Wolstenholme, 1989). In these tRNA genes, 12 mismatched base pairs are detected and all of them are G-U pairs. The anticodon (AC) arm of *trnSer2* forms two loops, which is uncommon in other lepidopteran species. The anticodons of the 22 tRNA genes of *E. grisescens* are all identical with other sequenced lepidopterans (Yang, Wei, Hong, Jiang, & Wen, 2009).

Two rRNA genes were identified in the conserved locations between *trnLeu1* and the control region of *E. grisescens*, with a total length of 2140 bp and an A+T content of 84.8% (Table 3). The large ribosomal RNA (*rrnL*) gene is 1375-bp long, with an A+T content of 84.5%. The small ribosomal RNA (*rrnS*) gene is 765-bp in size with an A+T content of 85.4%.

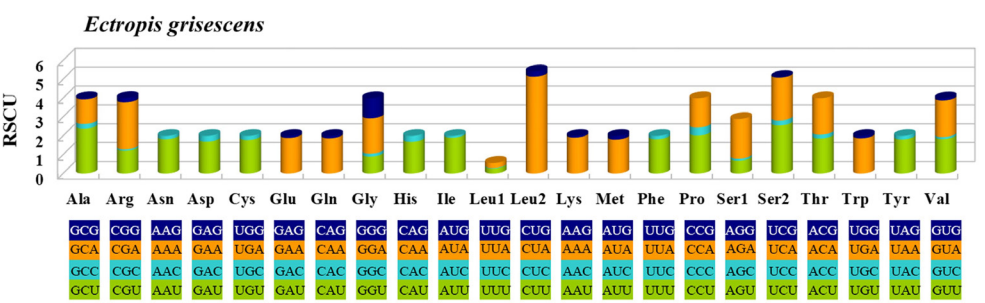

Fig. 2. Relative synonymous codon usage (RSCU) of PCGs in *Ectropis grisescens*. Full codon families are indicated below the X-axis.

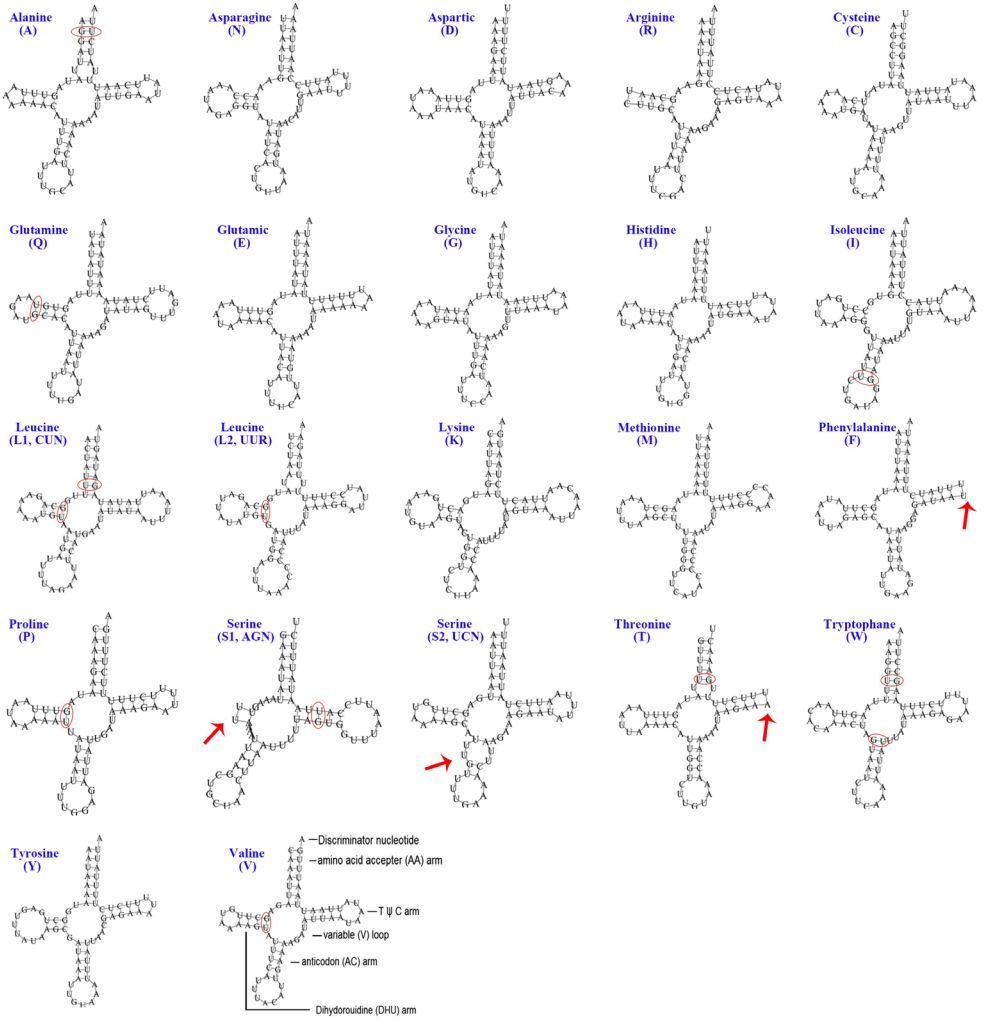

Fig. 3. Secondary structures of tRNA genes in the mitogenome of *Ectropis grisescens*. The tRNA genes are labelled with their corresponding amino acids. Structural elements are illustrated as for trnV. Modified arms are indicated with red arrows. Mismatched pairs are indicated with red circles.

Table 3. Nucleotide composition of the mitogenome of *Ectropis grisescens*.

Regions	A%	T%	G%	C%	A+T%	G+C%	AT Skew	GC Skew
Whole mitogenome	41.2	40.0	7.7	11.1	81.2	18.8	0.015	–0.181
PCGs	34.4	44.4	11.1	10.1	78.8	21.2	–0.127	0.047
1st codon position	36.3	44.6	9.7	9.4	80.9	19.1	–0.103	0.016
2nd codon position	34.3	44.5	11.9	9.3	78.8	21.2	–0.129	0.123
3rd codon position	32.6	44.1	11.6	11.7	76.7	23.3	–0.150	–0.004
PCGs-J	35.9	41.9	10.1	12.1	77.8	22.2	–0.077	–0.090
1st codon position	37.3	40.6	11.2	10.9	77.9	22.1	–0.042	0.014
2nd codon position	32.6	42.5	11.1	13.8	75.1	24.9	–0.132	–0.108
3rd codon position	37.7	42.5	8.1	11.7	80.2	19.8	–0.060	–0.182
PCGs-N	32.0	48.5	12.5	7.0	80.5	19.5	–0.205	0.282
1st codon position	43.6	39.7	6.7	10.0	83.3	16.7	0.047	–0.198
2nd codon position	38.9	42.8	5.5	12.8	81.7	18.3	–0.048	–0.399
3rd codon position	41.1	45.0	3.3	10.6	86.1	13.9	–0.045	–0.525
tRNA genes	41.6	40.0	8.3	10.1	81.6	18.4	0.020	–0.098
tRNA genes-J	41.9	38.6	10.1	9.4	80.5	19.5	0.041	0.036
tRNA genes-N	41.2	42.5	5.2	11.1	83.7	16.3	–0.016	–0.362
rRNA genes	43.9	40.9	4.9	10.3	84.8	15.2	0.035	–0.355
Control region	41.4	50.5	3.0	5.1	91.9	8.1	–0.099	–0.259

Control region

The longest non-coding sequence of the *E. grisescens* mitogenome is the control region located between *rrnS* and *trnMet* as in other lepidopterans (Fig. 1, Table 2). The control region of *E. grisescens* is 1550-bp in size, longer than all sequenced control regions of Geometridae. The A+T content of the control region is 91.9%, which is very high but still lower than *trnGlu* (92.3%).

As the most variable region in a mitogenome, the control region of *E. grisescens* contains some secondary structures (Fig. 4): a poly-T leading stretch (15,069-15,088) near the 5' end; seven complete and a partial tandem repeats (15,095-16,502) with a consensus size of 192 bp; three continuous stem-loop (SL) structures (16510-16575) near the 3′ end of the control region. The large tandem repeat region is the main reason for the large size of the control region in *E. grisescens*. The tandem repeats and SL structures can act as regulators during the mitogenomic replication and transcription processes (Crozier & Crozier, 1993). However, exact functions of the control regions of Lepidoptera are still unclear.

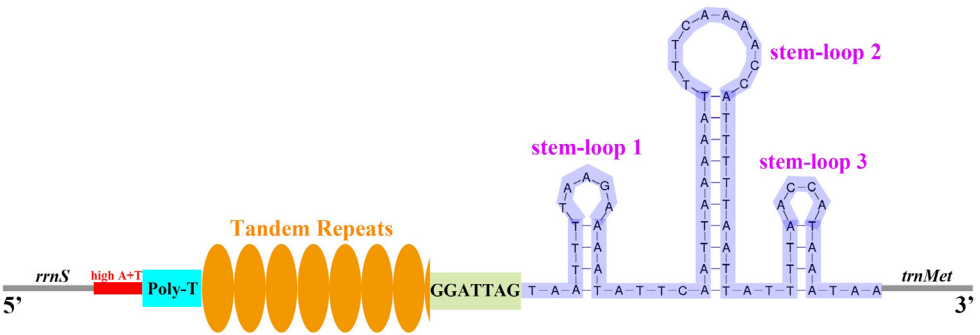

Fig. 4. Predicted structural elements in the control region of *Ectropis grisescens*. Poly-T stretch is indicated with blue box; tandem repeat units are indicated by orange ellipses; stem-loop structures are depicted showing their nucleotides. Other sequences with high A+T content are shown as red lines.

Phylogenetic analyses

The two phylogenetic trees respectively generated by the BI and ML analyses have almost identical topological structures (Figs. 5-6). The overall generic relationships recovered by both phylogenetic inferences are (((((*Biston* + (*Apocheima* + *Jankowskia*)) + *Ectropis*) + *Abraxas*) + *Celenna*) + *Phthonandria*) + (*Dysstroma* + *Operophtera*).

In both trees, the two subfamilies Ennominae and Larentiinae are recovered as monophyletic with high support (posterior probability = 1, bootstrap value = 100). The four species of genus *Biston* are grouped together, but *B. panterinaria* is clustered with *B. thibetaria* in the BI tree and with *B. perclara* in the ML tree. In *Ectropis*, *E. grisescens* sequenced herein is correctly supported as the sister group of *E. obliqua*. The sister group relationships are recovered between genera *Apocheima* and *Jankowskia*, and also between *Dysstroma* and *Operophtera*. In Ennominae, *Phthonandria* is supported as the basal group of its subfamily, but this conclusion is only reliable before further mitogenomes of Ennominae are sequenced.

The phylogenetic results of this study are generally identical with previous mitochondrial phylogenetic studies (Liu, Xue, Cheng, & Han, 2014; Xu, Chen, Wang, Peng, & Li, 2016; Sun et al, 2017; Li, Wang, Chen, & Han, 2018; Zheng et al, 2018). The mitogenomic data has been widely used in Lepidoptera during recent years and considerable efforts have been spent on the molecular phylogeny of diverse lepidopterans, however, very few attention was paid for Geometridae, leaving only nine genera with sequenced mitogemones, which is far from inferring a robust mitochondrial phylogeny of Geometridae. Additional sampling and mitogenome sequencing of each geometrid genus are vital works to clarify the phylogeny and evolution of this biodiverse and economically important insect group.

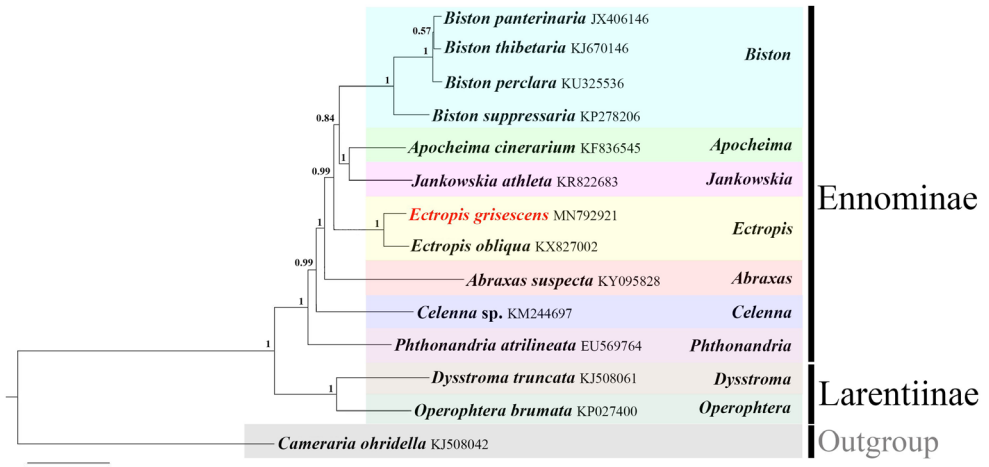

Fig. 5. Phylogenetic relationships within Geometridae inferred by Bayesian inference. Numbers at the nodes are posterior probabilities. The subfamily names are listed after the species. The tree was rooted with the outgroup *Cameraria ohridella*.

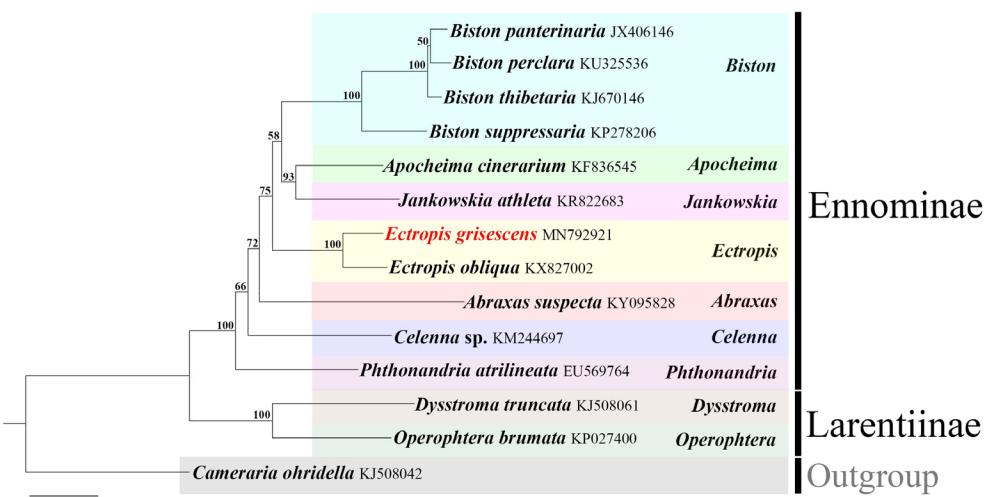

Fig. 6. Phylogenetic relationships within Geometridae inferred by maximum likelihood analysis. Numbers at the nodes are bootstrap values. Subfamily names are shown after the species. The tree was rooted with the outgroup *Cameraria ohridella*.

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REFERENCES

Burla, H. (1954). Zur Kenntnis der Drosophiliden der Elfenbeinkuste (Franzosisch West-Afnka). *Revue Suisse de Zoologie*, 61, 1-218.

Boore, J.L. (1999). Animal mitochondrial genomes. *Nucleic Acids Research*, 27, 1767-1780.

Bernt, M., Donath, A., Jühling, F., Externbrinka, F., Florentz, C., Fritzsch, G., Pütz, J., Middendorf, M., & Stadler, P.F. (2013). MITOS: Improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution*, 69, 313-319.

Crozier, R.H. & Crozier, Y.C. (1993). The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics*, 133, 97-117.

Chen, Z.T. & Du, Y.Z. (2016). Rearrangement of mitochondrial genome in insects. *Journal of Environmental Entomology*, 38, 843-851.

Ge, C.M., Yan, K.S., & Xiao, Q. (2015). Biological characteristic of *Ectropis grisescens* (Lepidoptera: Geometridae). *Annual Academic Conference of China Society of Plant Protection*, 522.

Grant, J.R. & Stothard, P. (2008). The CGView server: a comparative genomics tool for circular genomes. *Nucleic Acids Research*, 36, 181-184.

Garey, J.R. & Wolstenholme, D.R. (1989). Platyhelminth mitochondrial DNA: evidence for early evolutionary origin of a *tRNAserAGN* that contains a dihydrouridine arm replacement loop, and of serine-specifying AGA and AGG codons. *Journal of Molecular Evolution*, 28, 374-387.

Gu, P.W. & Xin, M. (2018). *Grape pathology and entomology*. Yangguang Press, Yinchuan, China.

Hebert, P.D.N., Cywinska, A., Ball, S.L., & deWaard, J.R. (2003). Biological identifications through DNA Barcodes. *Proceedings of the National Academy of Sciences of the United States of America*, 270, 313-321.

Jiang, N., Liu, S.X., Xue, D.Y., Tang, M.J, Xiao, Q., & Han, H.X. (2014). External morphology and molecular identification of two tea geometrid moth from southern China. *Chinese Journal of Applied Entomology*, 51, 987-1002.

Katoh, K. & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology Evolution*, 30, 772-780.

Li, Q., Wang, X., Chen, X., & Han, B. (2018). Complete mitochondrial genome of the tea looper caterpillar, *Ectropis obliqua* (Lepidoptera: Geometridae) with a phylogenetic analysis of Geometridae. *International Journal of Biological Macromolecules*, 114, 491-496.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., & Calcott, B. (2016). PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology Evolution*, 34, 772-773.

Luo, R.B., Liu, B.H., Xie, Y.L., Li, Z.Y., Huang, W.H., Yuan, J.Y., He, G.Z., Chen, Y.X., Pan, Q., Liu, Y.J., Tang, J.B., Wu, G.X., Zhang, H., Shi, Y.J., Liu, Y., Yu, C., Wang, B., Lu, Y., Han, C.L., Cheung, D.W., Yiu, S.M., Peng, S.L., Zhu, X.Q., Liu, G.M., Liao, X.K., Li, Y.R., Yang, H.M., Wang, J., Lam, T.W., & Wang, J. (2012). SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *GigaScience*, 1, 1-18.

Liu, S., Xue, D., Cheng, R., & Han, H. (2014). The complete mitogenome of *Apocheima cinerarius* (Lepidoptera: Geometridae: Ennominae) and comparison with that of other lepidopteran insects. *Gene*, 547, 136-144.

Mullen, G.R. & Zaspel, J.M. (2019). Moths and butterflies (Lepidoptera). In G.R., Mullen & L.A., Durden (Eds.). *Medical and veterinary entomology* (pp. 353-370). Elsevier, Amsterdam.

Perna, N.T. & Kocher, T.D. (1995). Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *Journal of Molecular Biology Evolution*, 41, 353-358.

Rambaut, A. & Drummond, A.J. (2019). Tracer Version 1.5. Retrieved from http://treebioedacuk/2019.

Ronquist, F. & Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572-1574.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and postanalysis of large phylogenies. *Bioinformatics*, 30, 1312-1313.

Simon, C., Buckley, T.R., Frati, F., Stewart, J.B., & Beckenbach, A.T. (2006). Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics*, 37, 545-579.

Scoble, M.J. (1999). *Geometrid moths of the world: A catalogue (Lepidoptera: Geometridae)*. CSIRO. Publishing, Collingwood, 1046 pp.

Stork, N.E. (2018). How many species of insects and other terrestrial arthropods are there on earth. *Annual Review of Entomology*, 63, 31-45.

Sun, Y., Zhang, J., Li, Q., Liang, D., Abbas, M.N., Qian, C., Wang, L., Wei, G.Q., Zhu, B.J., & Liu, C.L. (2017). Mitochondrial genome of *Abraxas suspecta* (Lepidoptera: Geometridae) and comparative analysis with other Lepidopterans. *Zootaxa*, 4254, 501-519.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology Evolution*, 30, 2725-2729.

Wei, S.J., Shi, M., Chen, X.X., Sharkey, M.J., van Achterberg, C., Ye, G.Y., & He, J.H. (2010). New views on strand asymmetry in insect mitochondrial genomes. *PLoS ONE*, 5, e12708, 10 pp.

Warren, W. (1894). News genera and species of Geometridae. *Novitates Zoologicae*, 1, 366-466.

Xu, Y.M., Chen, S.C., Wang, X.Q., Peng, P., & Li, P.W. (2016). The complete mitogenome of *Jankowskia athleta* (Lepidoptera: Geometridae). *Mitochondrial DNA Part A*, 27, 3035-3036.

Yang, L., Wei, Z.J., Hong, G.Y., Jiang, S.T., & Wen, L.P. (2009). The complete nucleotide sequence of the mitochondrial genome of *Phthonandria atrilineata* (Lepidoptera: Geometridae). *Molecular Biology Reports*, 36, 1441-1449.

Zheng, N., Sun, Y.X., Yang, L.L., Wu, L., Abbas, M.N., Chen, C., Gao, J., Li, X.K., Liu, C.L., & Dai, L.S. (2018). Characterization of the complete mitochondrial genome of *Biston marginata* (Lepidoptera: Geometridae) and phylogenetic analysis among lepidopteran insects. *International Journal of Biological Macromolecules*, 113, 961-970.

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