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Comparative analysis of the mitochondrial genome and genetic relationship between *Philosamia cynthia ricini* and *Philosamia cynthia cynthia*

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ABSTRACT: *Philosamia cynthia cynthia* and *Philosamia cynthia ricini* are silken insects belonging to the family Saturniidae (Lepidoptera). In order to explore the genetic relationships of silken insects from Saturniidae at the molecular level, we used NGS method to assemble complete mitochondrial genome sequences of *P. cynthia ricini* strain B7 and *P. cynthia cynthia* strain C2gx. Assembly results showed that the total lengths of the mitochondrial genome sequences of B7 and C2gx were 15 383 and 15 344 bp, respectively; and both genomes contained 37 genes with a A+T enriched region. A total of 361 transversion or transition sites occurred at 13 PCGs and 1 insertion or deletion site at the *Cox1*. The start codons of the *Cox1* in B7 and C2gx were CGA and ATT, respectively, which were different from the TTAG of other lepidopteran insects. Based on the mitochondrial genome sequences of fifteen lepidopteran insects published in NCBI, we performed phylogenetic analysis and showed that, although the two strains have some genetic differences in the mitochondrial genome, B7 and C2gx were clustered into one branch with close kinship as expected.

KEYWORDS: *Philosamia cynthia ricini*, *Philosamia cynthia cynthia*, mitochondrial genome, genetic relationship

INTRODUCTION

The mitochondrial genome (mt genome) is an important material for studying population genetics, genetics, and evolution due to its conserved gene arrangement, fast nucleotide substitution rate, and small genome size [1, 2]. In most animals, the mitochondrial DNA (mtDNA) has a closed double-stranded circular structure with length between 16–20 kb [3]. It generally contains 37 genes with a conserved gene order, which includes 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and 2 ribosomes RNA genes (rRNAs) [4].

In recent years, with the continuous development and improvement of the next generation sequencing (NGS) methods, related researches on mt genomes of plants and animals have also gradually expanded. For insects, mt genomes have been sequenced in various insects, such as Hemiptera, Lepidoptera, and Coleoptera, with some basic features compared, such as size, gene content, gene rearrangement, and codon usage, etc [5–7]. Using mt genome sequences, Jiang et al [8]; Hong et al [9]; and Feng et al [10] reconstructed the genetic relationship between different Lepidopteran species, and reached the same conclusions by using traditional classification methods. Song and Liang constructed a phylogenetic tree of Hemiptera based on 29 PCGs from the mt genome of multiple species, and revealed Hemiptera as a monophyletic taxon [11]. However, Cui et al [12] and Li et al [13] found some present systematic errors in the Hemiptera phylogenetic studies, due to effects of compositional heterogeneity. The above-mentioned cases proved that different evolution rates of the same genes between different species could result in the divergence of classification of species based on mt genome. Some highly conserved sequences of mtDNA are commonly used as DNA barcodes, and it has become an important method in species identification and classification. Common PCGs are used as DNA barcodes including the *Cytochrome C Oxidase Subunit 1* (*Cox1*) [14], *Cytochrome C Oxidase Subunit 2* (*Cox2*), and *NADH dehydrogenase*

subunit 5 (*Nad5*) [15].

Philosamia cynthia cynthia (*P. cynthia cynthia*) and *Philosamia cynthia ricini* (*P. cynthia ricini*) are both multivoltine (multiple life cycles a year) insects belonging to the lepidopteran family of Saturniidae. *P. cynthia cynthia* mainly feeds on *Ailanthus altissima (Mill.) Swingle* leaf with additionally feeding on *Ricinus communis L*. leaf, *Ilex chinensis Sims* leaf, and *Firmiana platanifolia (L.f.) Marsili* leaf, and diapauses in the form of pupa. Before, *P. cynthia cynthia* had been considered as a leaf-eating pest, but it has been used as a precious silk and edible insect resource in recent years in lieu of traditional control methods [16]. *P. cynthia ricini* is native to Assam, India, and was introduced to mainland China in 1940. *P. cynthia ricini* mainly feeds on *Ricinus communis L*. leaf, *Manihot esculenta Crantz* leaf, and *Ailanthus altissima (Mill.) Swingle* leaf. There is no diapause period under suitable conditions for *P. cynthia ricini*. Adults of both insects are similar in appearance, but the arrangement of scales on the abdomen is obviously different: horizontal in *P. cynthia cynthia*, while vertical in *P. cynthia ricini*. In additon, they can be crossed and the hybrids are fertile [17]. Among lepidopteran insects, *Bombyx mori* is the first species to have a complete mt genome sequenced. At present, there are many studies on sequencing and structural analysis of mt genomes of *B. mori*, *Antheraea assamensis*, etc., and completed mt genomes have been reported for *P. cynthia ricini* strains [18], but similar researches have been lacking for *P. cynthia cynthia*. Based on the basic composition structure and bases of *P. cynthia ricini* mt genome, studies of skewness, codon usage preferences, and kinship with other Saturniidae insects have provided data for genetic relationships of lepidopteran insects [19].

Comparative molecular characteristic studies have been done between the two silk insects of *B. mori* and *Antheraea proylei* using PCGs or rRNA [20]. However, comparative analyses of mt genome between *P. cynthia cynthia* and *P. cynthia ricini* are lacking. Here, we assembled complete mt genome sequences of *P. cynthia cynthia* and *P. cynthia ricini*. In addition, we contrasted the tRNA structural composition and the regions of PCGs in order to identify and analyze the differences of the two insects.

MATERIALS AND METHODS

Philosamia cynthia ricini strain B7 was collected from Xuzhou, Jiangsu Province. *Philosamia cynthia cynthia* strain C2gx was collected from Nanning, Guangxi Province. Both samples were preserved and stored in the Sericultural Research Institute, Chinese Academy of Agricultural Sciences.

Mitochondrial DNA was extracted by the classical "phenol-chloroform-isopentyl alcohol" method [21] from the male pupa of B7, and C2gx. DNA was fragmented using the Covaris machine. The DNA fragments were repaired at protruding ends using a combination of 3′–5′ exonuclease and polymerase, and a single base "A" was introduced at the 3′ of the segment to join the fragments with ligase to selectively enrich DNA fragments with joints at both ends while amplifying the DNA library [22]. Finally, fragment sizes were measured using the Agilent 2100 Bioanalyzer, and library concentration was detected by fluorescence quantification, followed by sequencing on the Illumina sequencing platform [23].

The SPAdes v3.9.0 [24] and A5-miseq v2015-0522 [25] softwares were used to assemble their high quality paired-end reads to construct contigs and scaffolding, respectively. Sequences with high sequencing coverage were extracted and compared with the nt library at NCBI using blastn (BLAST v2.2.31+) to select mitochondrial sequences of each spliced result. The obtained mitochondrial assembly results were first analyzed by mummer v3.1 [26] for collinearity and to determine the positional relationships between contigs, before gap filling between contigs was performed. The results were error-corrected using the Pilon v1.18 software [27] to obtain the final mitochondrial sequences. The assembled complete mt genome sequences were deposited at the MITOS web server for functional annotations [28].

The mt genome circle map was drawn with the cgview visualization software [29]. mt genome, PCGs, and rRNA base composition bias were calculated using formula: AT bias = (A−T)/(A+T); GC bias = (G−C)/(G+C) [30]. Through the calculation of relative synonymous codon usage (RSCU), the codon usages of B7 and C2gx were compared, and the coding protein sequences were analyzed.

ClustalW was used for the multiple sequence alignment of complete mt genome sequences from 15 lepidopteran insects published at NCBI. The phylogenetic tree was constructed using the MEGA 7.0 software by setting up the unrooted neighbor-joining (NJ) method with following parameters: 1000 times bootstrap value, poisson correction, and pairwise deletions [31].

RESULTS

Comparison of mitochondrial genome composition, structure and characteristics

The total lengths of mt genomes of B7 and C2gx were 15 383 and 15 344 bp, respectively. Both genomes contained 13 PCGs, 2 rRNAs, 22 tRNAs, and an A+T-rich region (OH region) (241 bp in B7 and 250 bp in C2gx). The gene orders of both mt genomes were the same as that of a typical lepidopteran mt genome, with a double-stranded (sense and antisense sequences) closed circular structure. For both genomes, there were 14 genes located in the antisense sequence, including 4 PCGs, 8 tRNAs, and 2 rRNAs. There were 23 genes located in the sense sequence, including 9 PCGs, 14 tRNAs, and 1 OH region (Figs. 1 and 2 and Table 1).

The total lengths of the 13 PCGs were 11 164 bp and 11 173 bp in B7 and C2gx, respectively. In B7, the *Cox1* uses CGA as the start codon, while the other PCGs use the start codon ATT/G/C; whereas in C2gx, all PCGs use the typical start codon ATT/G. The 12 PCGs in B7 and C2gx mt genomes use TAA as the stop codon, and only the *NADH dehydrogenase subunit 3* (*Nad3*) uses TAG as the stop codon. There were 8 and 9 overlapping gene pairs in B7 and C2gx, respectively. Base overlapping may be a way to reduce the sizes of the mt genomes.

The RSCU showed that the mt genomes of B7 and C2gx were both biased toward bases A and T, and the codons that start or stop with A and T were much more frequent than other synonymous codons.

Both mt genomes of B7 and C2gx contained 22 non-coding region sequences with lengths ranging from 1–250 bp. Among them, the OH region was 241 bp long in B7 and 250 bp long in C2gx. The OH region is the main regulatory region for replication and transcription of the mt genome [32]. Previous studies have indicated that there are repetitive sequences in the OH regions of lepidopteran mt genomes. The OH region of *Antheraea pernyi* contains a 38 bp repetitive unit [33], and $(AT)_n$ random repetitive sequences exist in the OH regions of *Antheraea yamamai*, *B. mori* and *Bombyx mandarina* [34]. In current study, the 38 bp repetitive unit was absent in the OH regions of both B7 and C2gx, but a repeat of $(AT)_{61}$ was found in B7 and a repeat of $(AT)_{59}$ in C2gx.

Both B7 and C2gx mt genomes contained 22 tRNAs and 2 rRNAs. The tRNA lengths were between 62–72 bp, rrnL lengths were 1295 (B7)/1289 bp (C2gx), and rrnS lengths were 763 (B7)/777 bp (C2gx). The tRNA of both genomes had some mismatched bases. Most of them were UG mismatched bases in typical tRNA secondary structure, and there were a few UU mismatched bases. There was only one pair of AA mismatched bases in tRNAs, which was not a typical secondary structure. In addtion, trnA, trnL2, trnN, trnS1, trnS2, trnT, and trnY did not have the typical secondary structure in B7 and trnA, trnL2, trnN, trnS1, trnS2 for C2gx (Figs. 3 and 4).

Mitochondrial protein coding sequences alignment and analysis of mutation sites

Basic local alignment of the entire mt genome sequences of B7 and C2gx showed that the similarity between the two genomes was 96.83%, indicating that the two subspecies are closely related. Insertions or deletions occurred at 23 local sites, and transversion or transition occurred at 454 sites. In order to further explore the compositions within PCGs, we compared 13 PCGs between B7 and C2gx one by one. The results revealed several gene mutations with varying degrees. The insertion or deletion of 13 PCGs only occurred at the start site of the *Cox1* in both B7 and C2gx (Fig. 5). A total of 361 transversions or transitions occurred between B7 and C2gx, which mainly concentrated within proteins such as *NADH dehydrogenase subunit 2* (*Nad2*), *Cox1*, *ATP synthase subunit 6* (*Atp6*), *Cytochrome C Oxidase Subunit 3* (*Cox3*), *Nad5*, *NADH dehydrogenase subunit 4* (*Nad4*), *NADH dehydrogenase subunit 1* (*Nad1*), and *Cytochrome B* (*Cob*). Among these PCGs, *Cox1* and *Nad5* are the greatest different PCGs between B7 and C2gx (Table 2 and Fig. 5). In addition, we compared the amino acid sequences and their secondary structures to analyze functional differences of the 13 PCGs. We found that except for *Nad4* and *NADH dehydrogenase subunit 4l* (*Nad4l*), the remaining 11 PCGs amino acid sequences were different at 2–13 sites with overall amino acid sequence similarity over 95%; and the secondary structures were identical. For *Nad4* and *Nad4l*, amino acid sequence similarity between the two subspecies was approximately 90%, and the differences in the secondary structure were also obvious.

Genetic relationship

We constructed phylogenetic tree using NJ method with complete mt genomic sequences of lepidopteran insects that are closely related to B7 and C2gx, including *A. yamamai*, *Actias luna*, *Actias*

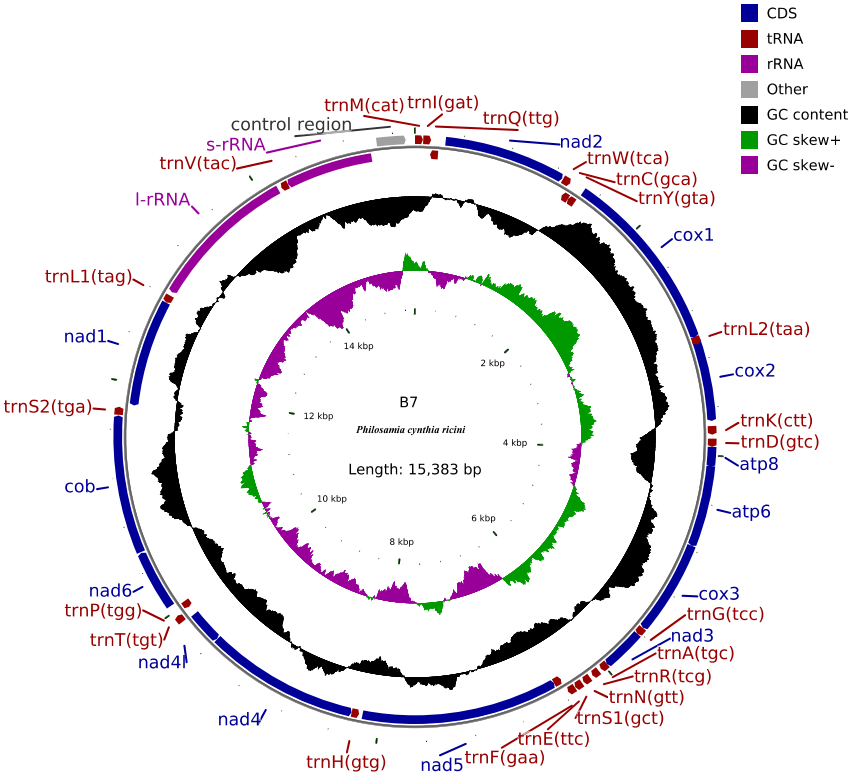


Fig. 1 B7 mt genome.

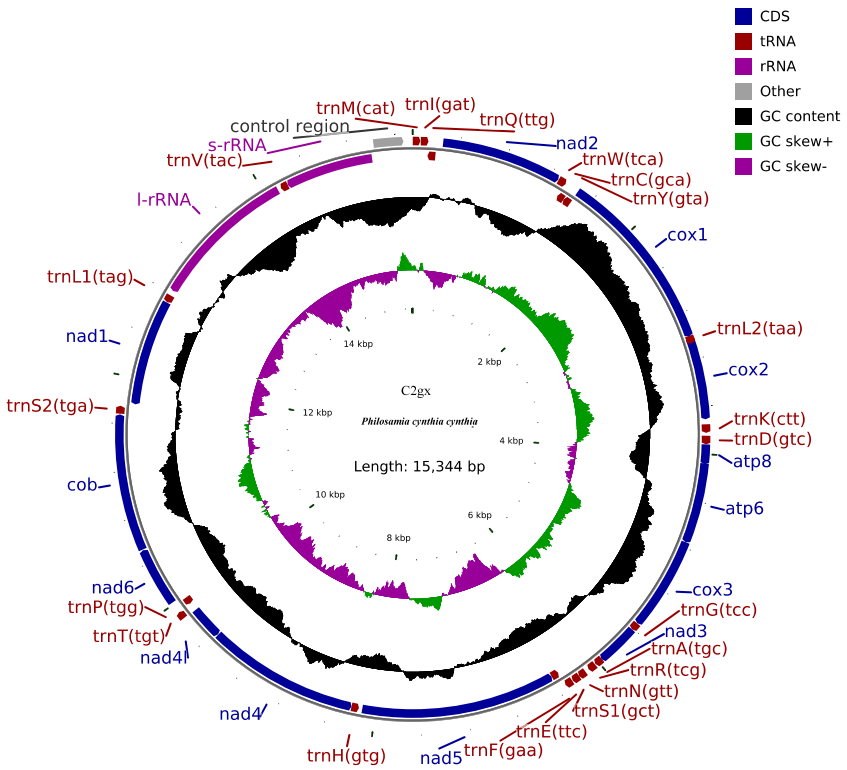


Fig. 2 C2gx mt genome.

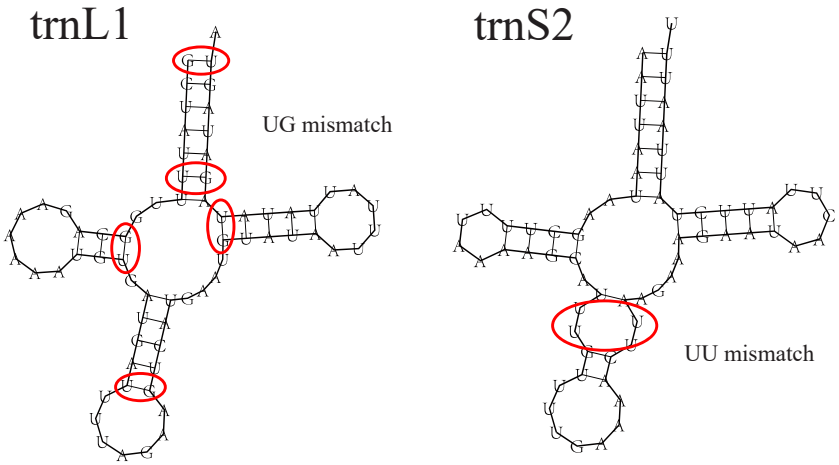


Fig. 3 B7 mt genome does not have a typical tRNA secondary structure (trnS2) and mismatched bases (in red circles).

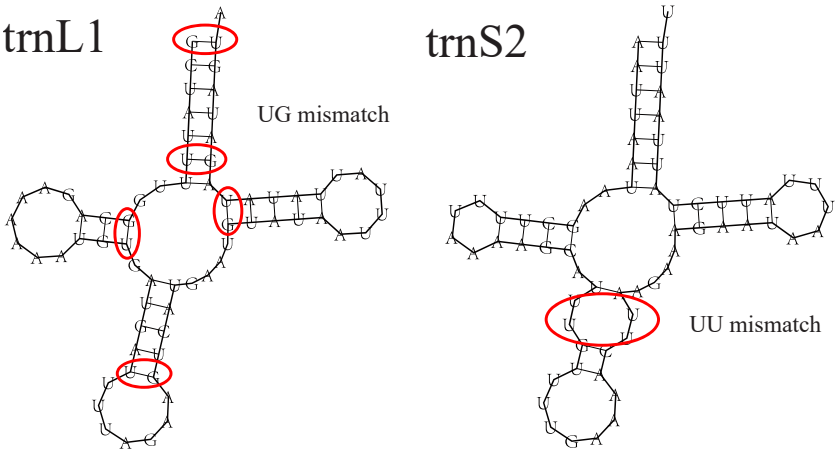
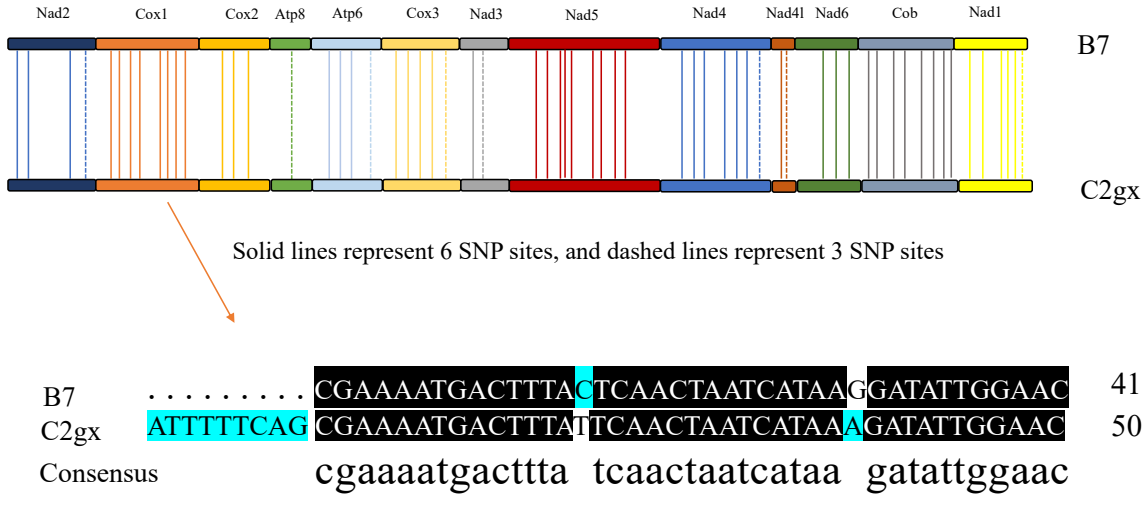


Fig. 4 C2gx mt genome does not have a typical tRNA secondary structure (trnS2) and mismatched bases (in red circles).



Indel region at the start of *Cox1* (partial sequences)

Fig. 5 Distribution of SNP and Indel sites in 13 PCGs.

Table 1 Characteristics of the mt genomes.

Name	Gene	Strand[a]	Start position	Size (bp)	Intergenic nucleotide[b]	Start codon	Stop codon	Anticodon
B7/C2gx	*trnM*	N	1	65/64	3			CAT
B7/C2gx	*trnI*	N	69/68	65	−3			GAT
B7/C2gx	*trnQ*	J	131	69	54/56			TTG
B7/C2gx	*nad2*	N	254/255	1014	9/3	ATT	TAA	
B7/C2gx	*trnW*	N	1277/1272	68	−8			TCA
B7/C2gx	*trnC*	J	1337/1332	62				GCA
B7/C2gx	*trnY*	J	1399/1394	65/66	9/0			GTA
B7/C2gx	*cox1*	N	1473/1460	1536/1545	−5	CGA/ATT	TAA	
B7/C2gx	*trnL2*	N	3004/3000	68	−45			TAA
B7/C2gx	*cox2*	N	3027/3023	688	42	ATG	T(AA)	
B7/C2gx	*trnK*	N	3757	71	33/24			CTT
B7/C2gx	*trnD*	N	3861/3848	68/72				GTC
B7/C2gx	*atp8*	N	3929/3920	165	−7	ATC/ATT	TAA	
B7/C2gx	*atp6*	N	4087	678	−1	ATG	TAA	
B7/C2gx	*cox3*	N	4764/4755	789	2	ATG	TAA	
B7/C2gx	*trnG*	N	5555/5546	66				TCC
B7/C2gx	*nad3*	N	5621/5612	354	−2	ATT	TAG	
B7/C2gx	*trnA*	N	5973/5964	66/67	24/5			TGC
B7/C2gx	*trnR*	N	6063/6036	64/68	26/32			TCG
B7/C2gx	*trnN*	N	6153/6136	65	14/−1			GTT
B7/C2gx	*trnS1*	N	6232/6200	66	4			GCT
B7/C2gx	*trnE*	N	6302/6270	66	−2			TTC
B7/C2gx	*trnF*	J	6366/6334	68/67				GAA
B7/C2gx	*nad5*	J	6434/6401	1716	30	ATT	TAA	
B7/C2gx	*trnH*	J	8180/8147	66	1			GTG
B7/C2gx	*nad4*	J	8247/8214	1341	3	ATG	TAA	
B7/C2gx	*nad4L*	J	9591/9558	291	12/8	ATG	TAA	
B7/C2gx	*trnT*	N	9894/9857	65/64				TGT
B7/C2gx	*trnP*	J	9959/9921	65	32			TGG
B7/C2gx	*nad6*	N	10056/10018	501	7/6	ATT	TAA	
B7/C2gx	*cob*	N	10564/10525	1152	5	ATG	TAA	
B7/C2gx	*trnS2*	N	11721/11682	66	24/25			TGA
B7/C2gx	*nad1*	J	11811/11773	939	2	ATG	TAA	
B7/C2gx	*trnL1*	J	12752/12714	68	33/44			TAG
B7/C2gx	*rrnL*	J	12853/12826	1295/1289	30			
B7/C2gx	*trnV*	J	14178/14145	66				TAC
B7/C2gx	*rrnS*	J	14244/14211	763/777	59			
B7/C2gx	*OH*	N	15066/15017	241/250	76/77			

[a] Strand: J is the antisense strand and N is the sense strand.
[b] Intergenic nucleotide: the length of the interval between this gene and the previous gene.

artemis aliena, *Saturnia jonasii*, *Neoris haraldi*, *Ernolatia moorei*, *Mustilizans hepatica*, *Rondotia menciana*, *Triuncina daii*, *Rotunda rotundapex*, *B. mandarina*, *Antheraea assama*, *Eriogyna pyretorum*, *A. pernyi*, and *B. mori*. The results indicated that *P. cynthia ricini*, and *P. cynthia cynthia* clustered into one branch as expected (Fig. 6). Meanwhile, the 3 *Antheraea* insects and the 2 *Bombyx* insects formed their own respective branches, reflecting their close relationships. *M. hepatica* was the most distant from all the other insects.

DISCUSSION

By comparing PCGs, the biggest difference we found was that the *Cox1* of B7 did not use the typical start codon ATT/G. In the lepidoptera mt genome, TTAG is usually used as the start codon for the *Cox1*, and no canonical is available in tRNA and start region of the *Cox1* [35]. Nevertheless, neither B7 nor C2gx had this characteristic. The start codon of *Cox1* was

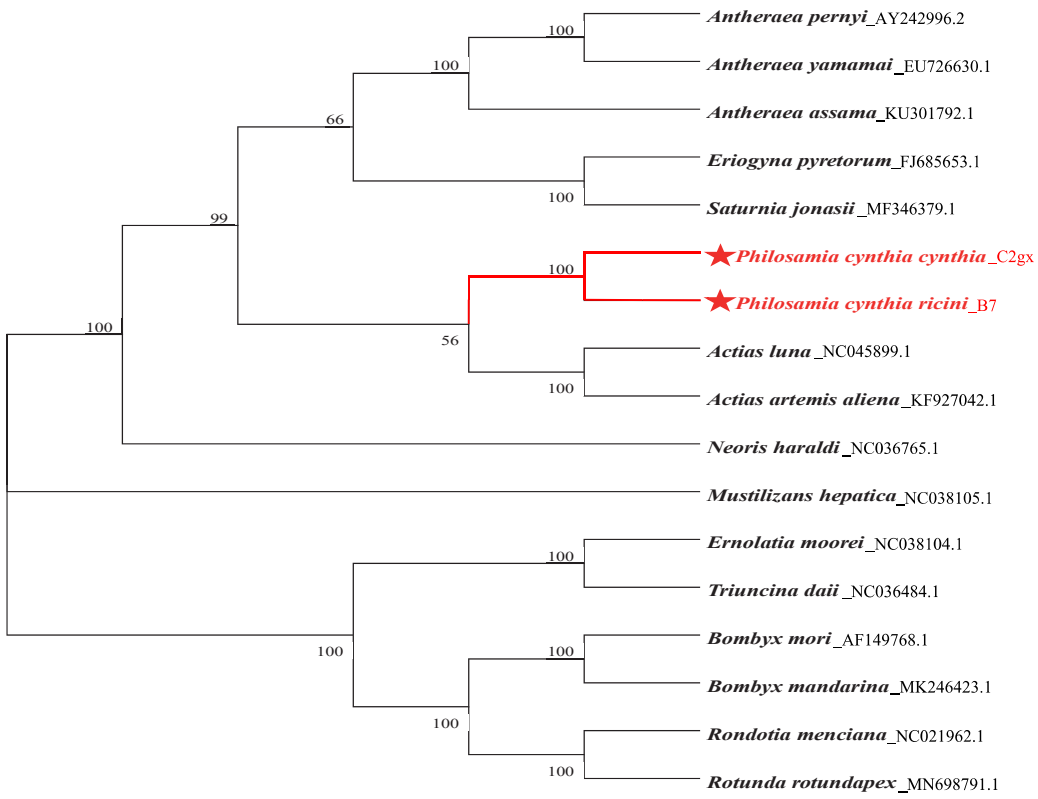


Fig. 6 Genetic relationships of 17 lepidopteran insects.

CGA and ATT distributed in B7 and C2gx, which code for arginine and isoleucine, respectively. In addition, the numbers of coding genes and their proportion in the total genome, A+T content, and AT bias were all similar in B7 and C2gx mt genomes.

In the non-coding regions, the biggest difference was that the OH region in C2gx was 9 bp longer than in B7. The two respectively contained $(AT)_{59}$ and $(AT)_{61}$ repeats and had a poly T structure with an ATAGA guide length of 19 bp. Based on previous researches, the origin of lagging strand replication in *Drosophila melanogaster* was related to (AT) repeats region [36]. In human mt genome, the change in the structure of tRNA was mapped to the origin of some lagging strands [37]. Thus, these sequences may play an important role in the transcription of their mitochondrial genes. And the start sequence of the OH region of C2gx was a 13 bp poly T structure, which was considered as the signal recognition site of the mt genome light chain replication initiation [38].

Table 2 Comparison of 13 coding gene mutation sites in B7 and C2gx.

Name	Gene	Bases	Indel	SNP
B7/C2gx	*Nad2*	1014	0	21
B7/C2gx	*Cox1*	1536/1545	9	49
B7/C2gx	*Cox2*	688	0	18
B7/C2gx	*Atp8*	165	0	3
B7/C2gx	*Atp6*	678	0	22
B7/C2gx	*Cox3*	789	0	29
B7/C2gx	*Nad3*	354	0	10
B7/C2gx	*Nad5*	1716	0	55
B7/C2gx	*Nad4*	1341	0	43
B7/C2gx	*Nad4l*	291	0	8
B7/C2gx	*Nad6*	501	0	20
B7/C2gx	*Cob*	1152	0	48
B7/C2gx	*Nad1*	939	0	35

For the comparison of tRNAs between B7 and C2gx, most of them had UG mismatched bases in tRNAs and the remaining are U-U mismatched. Both B7 and C2gx had a pair of AA mismatched bases in trnN. Base mismatches may affect tRNAs function; however, tRNAs may retain their normal functions through some modifications or sharing codons with neighboring downstream genes [37].

We also comparatively analyzed the mutation

sites in the PCGs. We found that there were 9 bases insertions or deletions in the *Cox1*. In addition, the *Cox1* could be used as a genetic marker among species in tapeworms, which was used to distinguish different strains of the same species [39] and applicable to B7 and C2gx. At the protein level, the differential expression of *Cox1* could be used to further differentiate the genetic relationship between B7 and C2gx. Besides, there were a few mutation sites in *Atp8* and *Nad4l*; while *Nad4/5*, *Cox1*, and *Cob* were also quite different due to the gene of transversion or transition. The *Nad* is relatively conserved in different taxa, but with high mutation rates among species, it was considered suitable as a genetic marker for species identification and diagnosis. The *Atp* played an important role in animal and plant energy metabolism and cell development. The *Cox* was crucial to growth, development, and energy utilization; while the *Cob* was greatly conserved and could be used as a potential genetic differentiation marker. In this study, we also analyzed the amino acid sequences and the secondary structures of the 13 PCGs. Whether the marked differences of *Nad4* and *Nad4l* in the composition and structure may be another important reason for the divergence in related functions and genetic evolution of B7 and C2gx needs further experiments.

CONCLUSION

In this study, we used NGS method to assemble the mt genomes of B7 and C2gx to analyze the whole mt genome, OH regions, tRNAs, and PCGs. The results indicated that there were indeed some mutation sites and different aspects in B7 and C2gx, and these could be related to genetic relationship. Finally, by constructing a phylogenetic tree using the NJ method and based on complete mt genomic sequences of 17 lepidopteran insects, we found that B7 and C2gx shared a high degree of genetic relationship as expected.

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REFERENCES

1. Cheng XY, Zhou HZ, Zhang GX (2000) Application of molecular biology technology in insect systematic research. *Zool Syst* **2**, 121–133.
2. Li G, Qian HY, Zhang YH, Zhao GD, Xu AY (2019) The complete mitochondrial genome and phylogenetic analysis of the *Philosamia cynthia ricini* 754. *Mitochondrial DNA B Resour* **4**, 132–133.
3. Boore JL (1999) Animal mitochondrial genomes. *Nucleic Acids Res* **27**, 1767–1780.
4. Boore JL, Medina M, Rosenberg LA (2004) Complete sequences of the highly rearranged molluscan mitochondrial genomes of the Scaphopod Graptacme eborea and the Bivalve Mytilus edulis. *Mol Biol Evol* **21**, 1492–1503.
5. Guo ZL, Yuan ML (2016) Research progress on mitochondrial genomics of Hemiptera insects. *Sci China Life Sci* **46**, 151–166.
6. Wang W, Meng ZQ, Shi FX, Li FB (2013) Progress in comparative mitochondrial genomics of Lepidoptera insects. *Chin Sci Bull* **58**, 3017–3029.
7. Nie R, Yang XK (2014) Research progress on the mitochondrial genome of coleoptera insects. *Acta Ent Sin* **57**, 860–868.
8. 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 Bio Sci* **5**, 351–365.
9. Hong MY, Lee EM, Jo YH (2008) Complete nucleotide sequence and organization of the mitochondrial genome of the silk moth *Caligula boisduvalii* (Lepidoptera:Satuiniidae) and comparison with lepidoptera insects. *Gene* **413**, 49–57.
10. Feng X, Liu D, Wang N (2010) The mitochondrial genome of the butterfly *Papilio xuthus* (Lepidoptera:Papilionidae) and related phylogenetic analyses. *Mol Biol Rep* **37**, 3877–3888.
11. Song N, Liang A (2009) Complete mitochondrial genome of the small brown planthopper, *Laodelphax striatellus* (Delphacidae:Hemiptera), with a novel gene order. *Zool Sci* **26**, 851–860.
12. Cui Y, Xie Q, Hua J, Dang K, Zhou J, Liu X, Bu W (2013) Phylogenomics of Hemiptera (Insecta:Paraneoptera) based on mitochondrial genomes. *Syst Entomol* **38**, 233–245.
13. Li H, Shao R, Song N, Song F, Jiang P, Li Z, Cai W (2015) Higher-level phylogeny of paraneopteran insects inferred from mitochondrial genome sequences. *Sci Rep* **1**, 8527–8527.
14. Li Q, Li D, Ye H, Liu X, Shi W, Cao N, Duan Y (2011) Using COI gene sequence to barcode two morphologically alike species: the cotton bollworm and the oriental tobacco budworm (Lepidoptera:Noctuidae). *Mol Biol Rep* **38**, 5107–5113.
15. Albre J, Gers C (2008) Molecular phylogeny of the Erebia tyndarus (Lepidoptera, Rhopalocera, Nymphalidae, Satyrinae) species group combining CoxII and ND5 mitochondrial genes: A case study of a recent radiation. *Mol Phylogenet Evol* **47**, 196–210.
16. Du ZJ, Wang JY, Zhang XS (2008) The starting temperature and effective accumulated temperature of *Philosamia cynthia cynthia* eggs. *Acta Seric Sin* **34**, 351–353.
17. Li SY, Du ZJ (2014) The wild silkworm of the silk-

worm series–*Philosamia cynthia cynthia*. *China Seric* **34**, 92–94.
18. Zhong J, Liu ZH, Yang WK, Zhu F, Dong ZP (2017) Sequencing and sequence analysis of mitochondrial genome of *Antheraea assama*. *Acta Ent Sin* **60**, 936–949.
19. Yang SY, Sun W, Shen YH, Chen YP, Zhang Z (2014) Sequencing of mitochondrial genome of *Philosamia cynthia ricini* sequence structure characteristics and phylogenetic analysis. *Acta Seric Sin* **40**, 0429–0439.
20. Arunkumar KP, Metta M, Nagaraju J (2006) Molecular phylogeny of silkmoths reveals the origin of domesticated silkmoth, *Bombyx mori* from *Chinese Bombyx mandarina* and paternal inheritance of *Antheraea proylei* mitochondrial DNA. *Mol Phylogenet Evol* **40**, 419–427.
21. Tamura K, Aotsuka T (1988) Rapid isolation method of animal mitochondrial DNA by the alkaline lysis procedure. *Biochem Genet* **26**, 815–819.
22. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben, LA, Rothberg JM (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**, 376–380.
23. Mardis ER (2013) Next-generation sequencing platforms. *Rev Anal Chem* **6**, 287–303.
24. Schubert M, Lindgreen S, Orlando L (2016) AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Res Notes* **9**, ID 88.
25. Coil D, Jospin G, Darling AE (2015) A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. *Bioinformatics* **31**, 587–589.
26. Robert C (2004) Edgar, MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792–1797.
27. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Earl AM (2014) Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLos One* **9**, e112963.
28. Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, Fritzsch G, Stadler PF (2013) MITOS: improved *de novo* metazoan mitochondrial genome annotation. *Mol Phylogenet Evol* **69**, 313–319.
29. Stothard P, Wishart DS (2005) Circular genome visualization and exploration using CGView, *Bioinformatics* **21**, 537–539.
30. Perna NT, Kocher TD (1995) Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *J Mol Evol* **41**, 353–358.
31. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* **33**, 1870–1874.
32. Taanrnan JW (1999) The mitochondrial genome: structure, transcription, translation and replication. *Biochim Biophys Acta* **1410**, 103–123.
33. Liu Y, Li Y, Pan M, Dai F, Zhu X, Lu C, Xiang Z (2008) The complete mitochondrial genome of the Chinese oak silkmoth, *Antheraea pernyi* (Lepidoptera:Saturniidae). *Acta Biochim Biophy Sin* **40**, 693–703.
34. Pan M, Yu Q, Xia Y (2008) Characterization of mitochondrial genome of Chinese wild mulberry silkworm, *Bombyx mandarina* (Lepidoptera:Bombycidae). *Sci China Life Sci* **51**, 693–701.
35. Kim SR, Kim MI, Hong MY, Kim KY, Kang PD, Hwang JS, Kim I (2009) The complete mitogenome sequence of the Japanese oak silkmoth, *Antheraea yamamai* (Lepidoptera:Saturniidae). *Mol Biol Rep* **36**, 1871–1880.
36. Goddard JM, Wolstenholme DR (1980) Origin and direction of replication in mitochondrial DNA molecules from the genus Drosophila. *Nucleic Acids Res* **8**, 741–757.
37. Kirino Y, Yasukawa T, Ohta S, Akira S, Ishihara K, Watanabe K, Suzuki T (2004) Codon-specific translational defect caused by a wobble modification deficiency in mutant tRNA from a human mitochondrial disease. *Proc Natl Acad Sci USA* **101**, 15070–15075.
38. Saito S, Tamura K, Aotsuka T (2005) Replication origin of mitochondrial DNA in insects. *Genetics* **171**, 1695–1705.
39. Arriaga JA, Roy L (2015) Co1 DNA supports conspecificity of *Geomyphiluspierai* and *G. barrerai* (Coleoptera, Scarabaeidae, Aphodiinae) and is a good marker for their phylogeographic investigation in Mexican mountains. *ZooKeys* **512**, 77–88.