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Mitochondrial Genomes of Lepidopteran Insects Considered Crop Pests

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Abstract

In this chapter, the complete mitochondrial genome of Guatemalan potato moth, *Tecia solanivora* (Povolny, 1973) (Lepidoptera: Gelechiidae) is presented as a model to understand how to characterize and study a mitogenome in insects. It was sequenced, analyzed, and compared with other lepidopteran insects. *T. solanivora* mitogenome is a circular double-stranded molecule, typically found in insects and containing 37 genes, all them well described over the other lepidopteran mitogenomes sequenced. Interestingly, in this mitogenome was found a gene arrangement in the tRNA-Met gene different from the ancestral arrangement, but commonly present in insect mitogenomes. Other important characteristics are the high A + T-biased and negative AT- and GC-skews contents, but also unusual canonical start codons in 12 protein-coding genes and an incomplete stop codon in the cytochrome oxidase subunit II gene consisting of just a Thymine. Another common feature shared with lepidopteran mitogenomes is the A + T-rich region. It is characterized by having 325 bb, the 'ATAGA' motif, a 17 bp poly (T) stretch and a (AT)₈ element preceded by the 'ATTTA' motif. Likewise, this mitogenome has 21 intergenic spacer regions. In addition, an update about other recent mitogenomes research done mainly over lepidopteran insects considered crop pests is presented. On the other hand, a novel development based on induced mutations by CRISPR-Cas9 in the mitogenomes seeking applicable capability for pest control is shown. The utility of this study is to improve scientific databases and support future studies of population genetic in lepidopteran.

Keywords: mitogenomes, mitochondrial genome, crop pests, lepidopteran, insects

1. Introduction

Crop loss is a function of one or more biotic factors, each of which may be contributing to a reduction in yield, whereas yield loss is the reduction in yield caused by a single pathogen or a

pest [1]. Even so, there is no doubt that crop losses due to pests and diseases are a major threat to incomes of rural families and to food security worldwide [2]. Although there are a large number of different living organisms that affect agricultural crops (biotic factors) and therefore they are called pests or pathogens, organisms from Lepidoptera order within Insecta class are considered one of the most economically important pests due to huge crop losses caused by them, crop losses, in terms of quantity and quality that can occur in the field (pre-harvest) or in the storage (post-harvest) [3].

Lepidoptera (moths and butterflies) is the second largest order in Insecta, is species-rich containing over 155,000 described species, and occurs in nearly all regions and a wide variety of habitats [4]. A combination of features has conspired to render the Lepidoptera one of the most studied groups of organisms; on account of this, research on lepidopteran insects has been carried out during the past century. Nevertheless, only few years ago, scientists are seeking answers on genomes as a key to revalidate previously generated data or redirect mechanisms of pest control. In this context, mitochondrial genomes (mtgenomes or mitogenomes) are very important subject for different scientific disciplines including, among others, animal health, comparative and evolutionary genomics, molecular evolution, phylogenetic, population genetics, and biogeographic studies [5, 6]. Therefore, it is not surprising that

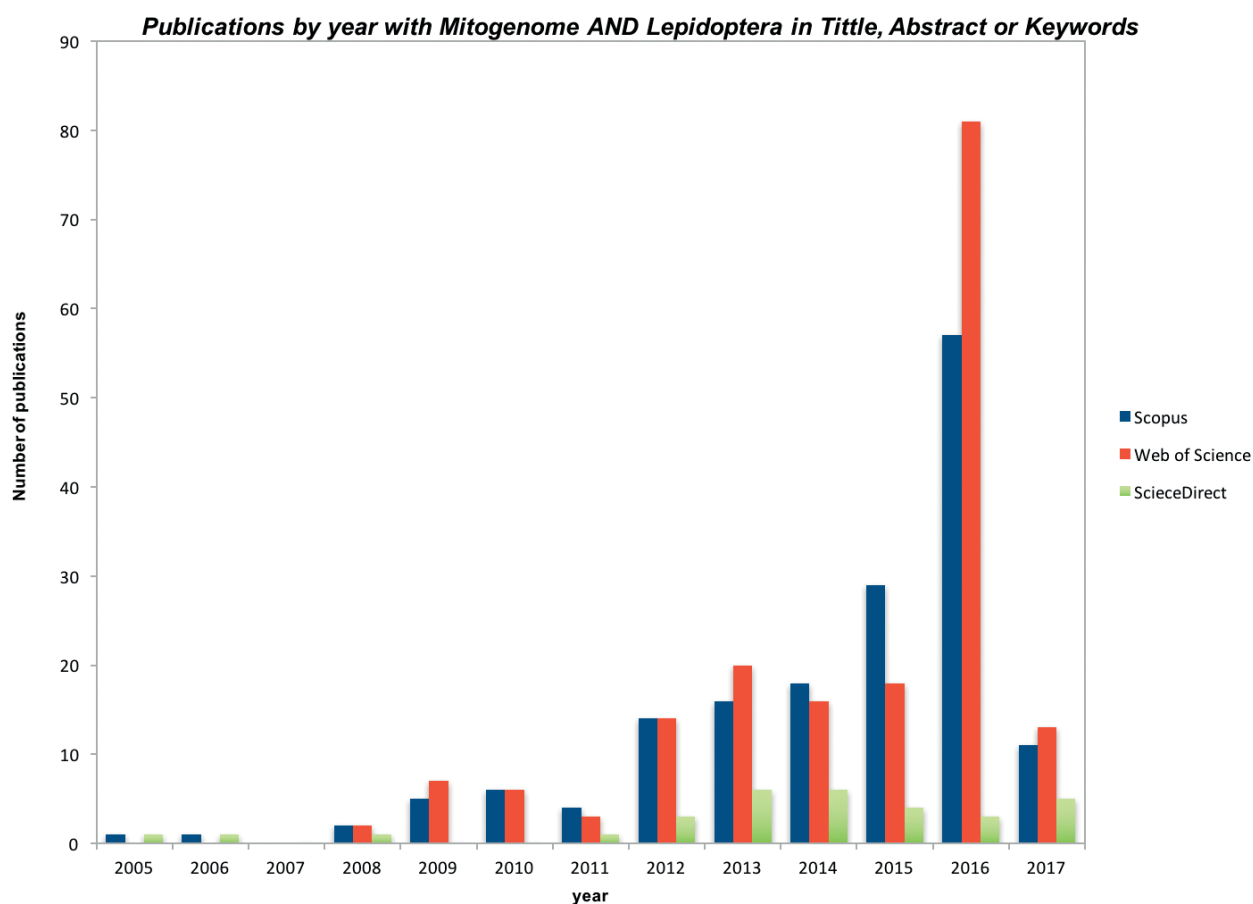


Figure 1. Growing in the number of publications with the words “Mitogenome AND Lepidoptera” at the title, abstract or keywords of scientific articles. Records were subtracted from literature databases; Scopus, Web of Science, and ScieceDirect.

approximately 500 mitogenomes of insects have been determined and subsequently deposited in GenBank [6]. Surprisingly, one of the most recent report shows that only 140 complete Lepidoptera mitogenomes (28 families from 12 superfamilies) have been sequenced and deposited in genomes databases [7], which contrasts with the number of described species in this order, as previously mentioned. In this perspective, it has been the growing research efforts of scientist around the world seeking to expand the knowledge barrier of one mitochondria of Lepidoptera. **Figure 1** shows continuous growth in the number of scientific publications in this field, showing records subtracted from literature databases: Scopus, Web of Science, and ScienceDirect.

In the present chapter, the complete mitogenome of *Tecia solanivora* is presented as a model to understand how to study a mitochondrial genome in insects. Additionally, it presented a review about mitogenomes research done mainly over lepidopteran insects considered crop pests to provide insight into aspects like genome structure and organization, nucleotide composition, codon usage, molecular functions, interactions among genes, and notable noncoding sequences included in the A + T-rich region. The utility of this study is to improve databases and support the determination of lepidopteran population genetic studies in the future.

2. Mitochondrial genome in insects

In insects, the mitochondrial genome is a circular double-stranded molecule typically between 14,000 and 20,000 bp. It contains 13 PCGs, 2 rRNAs, 22 tRNAs, and a control region (also known as the A + T-rich region), which are organized and oriented in different ways [8]. This genome has been widely used for phylogeny studies, phylogeography, population genetics, and molecular diagnostics. It has also been used to identify novel genes relevant for future studies [9], because of its small size, maternal inheritance, low recombination rate, relatively rapid evolutionary frequency, and multiple copies per cell [10]. Consequently, mitogenome sequences are rapidly evolving with about 500 insect species currently sequenced [6].

3. Characterization of insect mitogenomes

The complete mitogenome of *Tecia solanivora* is presented as a model to understand how to study and characterize a mitochondrial genome in insects. Additionally, it presented a review about mitogenomes research done mainly over lepidopteran insects considered crop pests. The argument for presenting *T. solanivora* as a model is because this lepidopteran insect represents the most damaging potato (*Solanum tuberosum*) pest in both Central and South America and Spain [11, 12]. *T. solanivora* was reported first time in Central America in 1956, affecting potato crops (*S. tuberosum*), which resulted in a direct effect on the economy. Even though this pest has a reduced mobility, it has invaded several countries in Central and South America as well as the Canary Islands in Spain where potato is grown [13]. It is important to mention that *T. solanivora* has been producing damage in both field crops and stored potato tubers, causing economic losses ranged from 50 to 100% [14]. The economic impact of the pest in countries of

the Andean area is much more serious than in Central America, mainly because potato is an important family staple and its production is very intensive. Therefore, *T. solanivora* is considered the most damaging crop insect pest in such countries [15]. Nevertheless, as an insect belonging to Lepidoptera order, the study carried out on the characterization of *T. solanivora* mitogenome could be applied to other studies on insects considered pest for agriculture.

3.1. Genome sequencing and assembling

T. solanivora larvae were collected from field- or storage-infested potato tubers from Colombia. All biological samples were preserved in 70% ethanol and stored at -70°C until DNA extraction. Whole *T. solanivora* genomic DNA was extracted using the protocol described by [13]. Each sample was analyzed by electrophoresis and the DNA concentration was quantified using a NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA). The whole genome was sequenced by Illumina Hiseq 2000 system at Chapel Hill High-Throughput Sequencing Facility in the University of North Carolina. The sequencing system generated 100 bp paired-end reads with a 342 bp insert size, these reads were checked and filtered using a homemade quality criterion script and finally the paired-end reads were assembled using de novo assembler VELVET 1.2.10 [16] with an optimized k-mer parameter of 99. The mtDNA was identified through a comparison between *Tecia solanivora* scaffolds and the mtDNA sequences reported in the NCBI GenBank, resulting in the identification of a single mtDNA contig with 200× coverage. To verify the topology of the mtDNA, the reads that mapped to the borders of the contig were located at an expected distance from their respective pairs. Any discrepancies that occurred, especially in the homopolymer regions, were manually edited.

3.2. Gene annotation and compositional analysis

To predict the protein-coding genes (PCGs), rRNA genes and tRNA genes from *T. solanivora* mtDNA, their sequence was submitted to the automatic annotator of mitochondrial genes online [dual organellar genome annotation (DOGMA), <http://dogma.cccb.utexas.edu>] [17]. To determinate homology between *T. solanivora* genes and other previously sequenced Lepidoptera species was used NCBI BLAST program and results manually curated. Then, the PCGs nucleotide composition, genome, and codon position were determined and the PCGs were translated into putative proteins for calculating of the relative synonymous codon usage (RSCU) using the invertebrate mitochondrial genetic code in MEGA version 5.2.2 [18]. The frequencies of A, T, G, and C were used to calculate the composition skew according to the AT- and GC- skew formulas. The intergenic and overlap sequences were pulled out manually from genome using SeqBuilder from the DNASTar package (DNASTar Inc., Madison, Wisconsin, USA).

3.3. Genome structure, organization, and base composition obtained

The *T. solanivora* mitogenome obtained was a closed circular 15,251-bp molecule (GenBank accession number KT326187) (**Figure 2**). It contains the typical set of 37 genes (13 PCGs, 22 tRNAs, and two rRNAs) and a large, 325-bp noncoding region (control region). A total of 24 genes were transcribed on the majority-coding strand (H-strand), while the rest were transcribed on the minority-coding strand (L-strand) (**Table 1**).

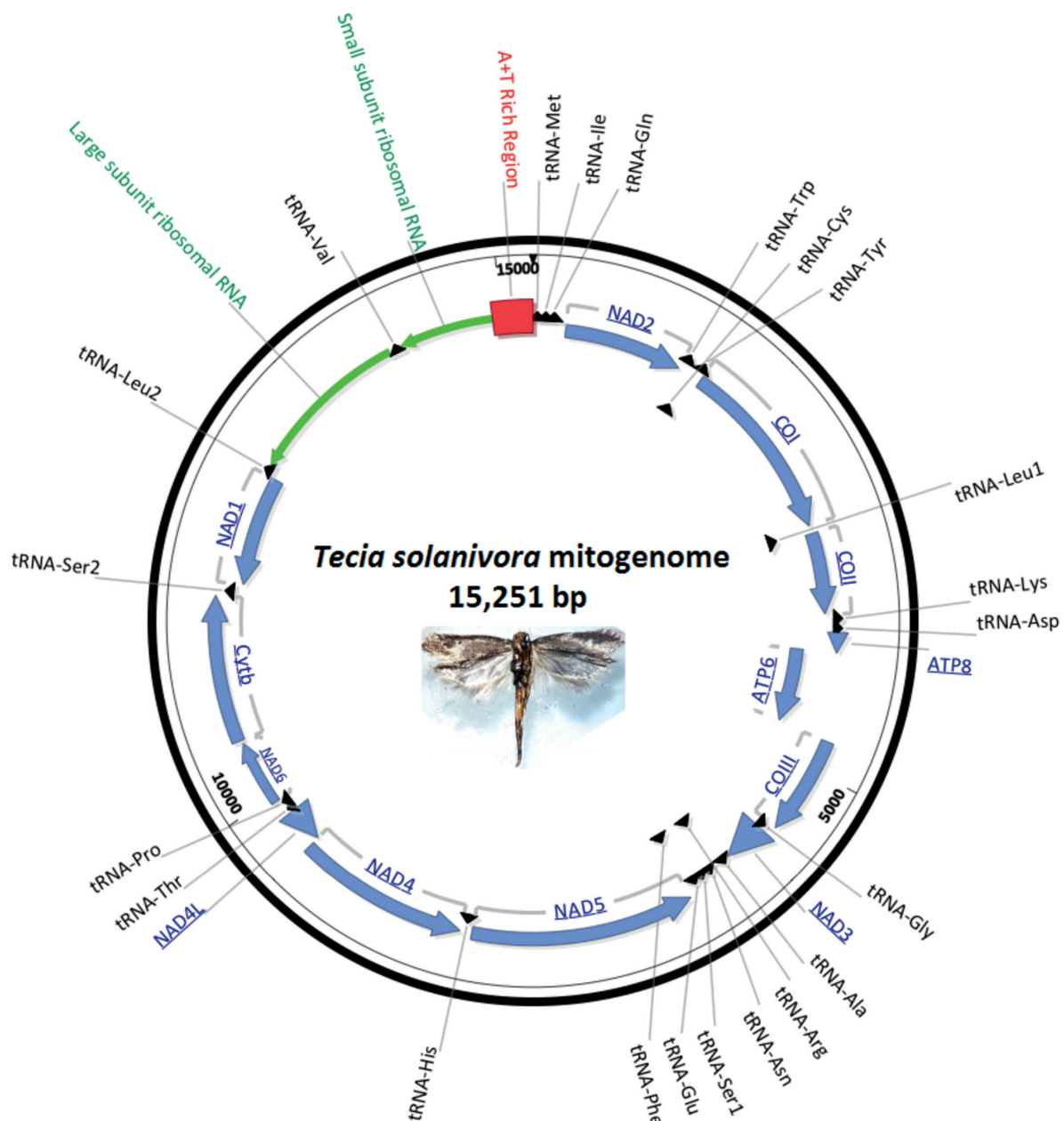


Figure 2. Map of the mitochondrial genome of *T. solanivora*. Protein-coding genes (names with underline) coded on the majority strand arrows going in clockwise direction, while the rest going counterclockwise. The tRNA genes are designated by tRNA-amino acid codes. The rRNAs two and they are located next to tRNA-Val and the A + T-rich region (control region) is indicated by a square.

When we compared with other reported Lepidoptera family mitogenomes, it found an identical gene order and orientation of the mitochondrial genes of this species to other lepidopteran moths, including *Tryporyza incertulas* [19], *Corcyra cephalonica* [20], *Adoxophyes honmai* [21], *Apocheima cinerarius* [22], *Amata emma* [23], *Attacus atlas* [24], *Bombyx mori* [25], *Caligula boisduvalii* [26], *Chilo auricilius* [19], *Diaphania pyloalis* [27], *Manduca sexta* [9], *Ostrinia nubilalis*, *Ostrinia furnacalis* [28], *Samia Cynthia ricini* [29], and *Sasakia funebris* [30], among others.

Gene	Direction	Position (bp)	Length (bp)	Anticodon	Start codon	Stop codon
<i>tRNA-Met</i>	Forward	1–68	68	CAT		
<i>tRNA-Ile</i>	Forward	70–134	65	GAT		
<i>tRNA-Gln</i>	Reverse	136–204	69	TTG		
<i>NAD2</i>	Forward	259–1269	1011		ATT	TAA
<i>tRNA-Trp</i>	Forward	1268–1336	69	TCA		
<i>tRNA-Cys</i>	Reverse	1329–1394	66	GCA		
<i>tRNA-Tyr</i>	Reverse	1406–1471	66	GTA		
<i>COI</i>	Forward	1475–3010	1536		CGA	TAA
<i>tRNA-Leu (UUR)</i>	Forward	3006–3073	68	TAA		
<i>COII</i>	Forward	3074–3754	681		ATG	T
<i>tRNA-Lys</i>	Forward	3756–3826	71	CTT		
<i>tRNA-Asp</i>	Forward	3837–3904	68	GTC		
<i>ATP8</i>	Forward	3905–4072	168		ATT	TAA
<i>ATP6</i>	Forward	4066–4743	678		ATG	TAA
<i>COIII</i>	Forward	4743–5531	789		ATG	TAA
<i>tRNA-Gly</i>	Forward	5534–5600	67	TCC		
<i>NAD3</i>	Forward	5601–5954	354		ATT	TAA
<i>tRNA-Ala</i>	Forward	5964–6030	67	TGC		
<i>tRNA-Arg</i>	Forward	6030–6095	66	TCG		
<i>tRNA-Asn</i>	Forward	6101–6166	66	GTT		
<i>tRNA-Ser (AGN)</i>	Forward	6181–6246	66	GCT		
<i>tRNA-Glu</i>	Forward	6247–6315	69	TTC		
<i>tRNA-Phe</i>	Reverse	6314–6380	67	GAA		
<i>NAD5</i>	Reverse	6364–8097	1734		ATT	TAA
<i>tRNA-His</i>	Reverse	8113–8178	66	GTG		
<i>NAD4</i>	Reverse	8183–9523	1341		ATG	TAA
<i>NAD4L</i>	Reverse	9523–9816	294		ATG	TAA
<i>tRNA-Thr</i>	Forward	9819–9883	65	TGT		
<i>tRNA-Pro</i>	Reverse	9884–9949	66	TGG		
<i>NAD6</i>	Forward	9952–10,479	525		ATA	TAA
<i>Cytb</i>	Forward	10,497–11,642	1146		ATA	TAA
<i>tRNA-Ser</i>	Forward	11,646–11,712	67	TGA		
<i>NAD1</i>	Reverse	11,730–12,665	936		ATA	TAG
<i>tRNA-Leu</i>	Reverse	12,669–12,736	68	TAG		

Gene	Direction	Position (bp)	Length (bp)	Anticodon	Start codon	Stop codon
<i>rRNA-Large</i>	Reverse	12,737–14,065	1329			
<i>tRNA-Val</i>	Reverse	14,089–14,155	67	TAC		
<i>rRNA-Small</i>	Reverse	14,157–14,926	770			
A + T region		14,927–15,251	325			

Table 1. Summary of *T. solanivora* mitogenome.

The typical lepidopteran arrangement of the tRNAs (tRNA-Met, tRNA-Ile, tRNA-Gln) was observed in the *T. solanivora* mitogenome but differs from the order found in ancient insects (**Figure 3**). In that sense, it was determined that *T. solanivora* presents several differences from the ancestral organization of the tRNA-Met region (A + T-rich region, tRNA-Ile, tRNA-Gln, tRNA-Met) [20], which is also found in the mitogenomes of *Aedes aegypti* (Diptera) [31] and *Acrida cinerea* (Orthoptera) [32]. In the case of *T. solanivora*, the order is: A + T region, tRNA-Met, tRNA-Ile, tRNA-Gln. Additionally, in the *T. solanivora* mitogenome, the tRNA-Lys gene is found after the COII gene, contrary to *A. cinerea*, where they are found in the reverse order. In addition, the NAD3 gene was located before the tRNA-Ala gene in *T. solanivora*, whereas in *A. aegypti*, the gene located in this region is tRNA-Arg.

The nucleotide composition determined in the entire *T. solanivora* mitogenome was A: 38.6, T: 39.6, C: 13.3, and G: 8.4% (**Table 2**). This nucleotide composition shows that highly A + T-biased (78.2%) with a similar proportion of adenine (A) and thymine (T) compared with the reported ranges found in other Lepidoptera mitogenomes. In the same way, *T. solanivora* mitogenome exhibits negative AT-skew (−0.013) and GC-skew (−0.226) values (**Table 3**). However, the most of Lepidoptera family members have shown higher percentages of A than T, such as: *O. nubilalis* (A: 41.3, T: 38.8%), *O. furnacalis* (A: 41.46, T: 38.92%), *B. mori* (A: 43.06, T: 38.30%) [33], *Phthonandria atrilineata* (A: 40.78, T: 40.24%) [34], *Ochrogaster lunifer* (A: 40.09, T: 37.75%) [35], *Chinese Bombyx mandarina* (A: 43.11, T: 38.48%) [36], and *A. atlas* (A: 39.8, T: 39.5%), among others. Likewise, the cytosine (C) content in the *T. solanivora* mitogenome was greater than guanine (G), which is similar to the percentages identified in other recently discovered Lepidoptera mitogenomes, except for *Antheraea yamamai* (G: 10.71, C: 10.35%) [37], *Eriogyna pyretorum* (G: 10.61, C: 7.45), and *Artogeia melete* (G: 11.33, C: 8.65%) [38].

3.4. Protein-coding genes (PCGs)

The protein-coding genes (PCGs) encompassed 11,191 bp of the entire assembled sequence (73.38%) and exhibited an A + T content of 76.4%. Nine of the 13 PCGs are coded on the majority strand (ATP6, ATP8, COI, COII, COIII, Cytb, NAD2, NAD3, and NAD6), while the rest (NAD1, NAD4, NAD4L, and NAD5) are coded on the minority strand. For the protein-coding genes, the A + T content was calculated for the three-codon positions, and they showed few differences from other Lepidoptera mitogenomes. *T. solanivora* showed negative AT- and GC-skew values in the second and third positions of each codon, indicating a greater inclination for the nitrogen bases, T and C, while the first position showed a slightly positive value

nt %	Whole mtDNA	Protein-coding sequence			Concatenated PCGs	rRNAs	tRNAs	IGs	A + T-rich region
		1st#	2nd#	3rd#					
A%	38.6	35.3	21.3	38.3	31.7	43.8	40.5	44.4	42.8
T%	39.6	36.6	48.2	49.2	44.7	39.9	40.2	44.4	48.3
C%	13.3	11.0	17.0	7.3	11.7	5.2	8.1	7.6	6.2
G%	8.4	17.7	13.5	5.2	11.9	11.1	11.2	3.5	2.8
A + T%	78.2	71.9	69.5	87.5	76.4	83.7	80.7	88.8	91.1
C + G%	21.7	28.7	30.5	12.5	23.7	16.3	19.3	11.1	9.0
AT-Skew%	-0.013	-0.018	-0.387	-0.125	-0.170	0.047	0.004	0	-0.060
GC-Skew%	-0.226	0.233	-0.115	-0.168	0.008	0.362	0.161	-0.36	-0.378

Species	Length (bp)	A%	G%	T%	C%	A + T%	G + C%	AT-skew	GC-skew
<i>T. solanivora</i>	15,251	38.6	8.4	39.6	13.3	78.2	21.7	−0.013	−0.226
<i>A. selene</i>	15,236	38.54	8.05	40.37	13.03	78.91	21.08	−0.023	−0.236
<i>C. raphaelis</i>	15,314	39.37	7.30	43.29	10.04	82.66	17.34	−0.047	−0.158
<i>E. pyretorum</i>	15,327	39.17	7.63	41.65	11.55	80.82	19.18	−0.031	−0.204
<i>A. yamamai</i>	15,338	39.26	7.69	41.04	12.02	80.30	19.71	−0.022	−0.220
<i>C. boisduvalii</i>	15,360	39.34	7.58	41.28	11.79	80.62	19.37	−0.024	−0.217
<i>S. cynthia ricini</i>	15,384	39.65	7.81	40.13	12.41	79.78	20.22	−0.006	−0.227
<i>M. sexta</i>	15,516	40.67	7.46	41.11	10.76	81.78	18.22	−0.005	−0.181
<i>A. pernyi</i>	15,566	39.22	7.77	40.94	12.07	80.16	19.84	−0.021	−0.217
<i>A. honmai</i>	15,680	40.15	7.88	40.24	11.73	80.39	19.61	−0.001	−0.196

Table 3. Comparison of nucleotide composition and skewness between *T. solanivora* and other lepidopteran mitogenomes.

for the GC-skew indicating a greater bias for G than C (**Table 3**). In general, the codons of *T. solanivora* mitogenome present high A + T content for the first position (71.9%). Similar values were observed in *A. emma* (73.1%), *Antheraea pernyi* (72.9%) [39], *C. boisduvalii* (73.8%), *B. mandarina* (75.0%) [33], and *M. sexta* (74.8%), but in the second position was found a lower A + T percentage (69.5%).

Twelve PCGs were identified in the *T. solanivora* mitogenome with the typical ATN initiation codons (isoleucine and methionine), except for the COI gene that is initiated by CGA initiation codon (arginine). The typical ATN codon represents a putative codon commonly observed in the order Lepidoptera [9, 40], and is thus considered a synapomorphy of this group of insects [41]. The methionine start codon, ATG, was used by five of the 13 PCGs, and ATA was used to initiate protein synthesis in the *NAD1*, *NAD6*, and *cytb* genes. In contrast, an atypical isoleucine codon (ATT) was used to initiate protein synthesis in the *ATP8*, *NAD2*, *NAD3*, and *NAD5* genes. Arginine (CGA) was used for the COI gene for which a nucleotide sequence of four or six base pairs has been proposed, much like TTAG in *Maruca vitrata* [42], to serve in a nonstandard initiation process located immediately upstream from the putative arginine CGA start codon of COI. In *T. solanivora*, this tetranucleotide sequence consisted of TTGG. The high A + T percentages in insect mitogenomes result in high probabilities of finding a noncoding triplet or a coding triplet within the tRNA-Tyr gene, a result that could potentially produce generalized annotation errors for the gene COI [43]. Previous studies have discussed the possibility that translation initiation of this gene involves an unusual sequence of four to six nucleotides (ATAA, TTAA, GTAA, ATTA, or ATTTAA) located immediately before the coding primer of the COI gene. This sequence apparently functions as the translation initiator in the majority of insects from the family Diptera, including *Drosophila yakuba* [44]. However, in *T. solanivora*, the sequence TTGG was found immediately before putative initiation codon CGA.

For the stop codon genes, we found the TAA codon in 11 of the PCGs, coinciding with the mitogenomes of other Lepidoptera, including *T. incertulas*, *S. funebris*, *S. cynthia*, and *A. emma* of the family Hesperidae. In the *NAD1* gene, the TAG stop codon was found and similar results were obtained in five species of the family Hesperidae [41], while the *COII* gene used a single T as an incomplete stop codon, which is commonly found in the majority of Lepidoptera species to date [9, 41]. This truncated codon could be a representative of a recognition site for an endonuclease that splits the polycistronic pre-mRNA, where a post-transcriptional polyadenylation then occurs, resulting in a functional stop codon (TAA) [9, 19, 45].

The CDpT or Codons Per Thousand Codons of the *T. solanivora* mitogenome was calculated, and five amino acid families were identified. The most common families were: phenylalanine (Phe), asparagine (Asn), isoleucine (Ile), lysine (Lys), and leucine 2 (Leu 2), as shown in (**Figure 4a**), being in *T. solanivora*, phenylalanine (Phe) the most abundant, instead of Leucine 2 (Leu2), which dominates in other Lepidoptera mitogenomes [35]. Additionally, when the relative synonymous codon use (RSCU) was determined, we identified that *T. solanivora* presents all typical codons found in other invertebrates (**Figure 4b**). The codons were richer in A or T at the third position and consequently have less G or C.

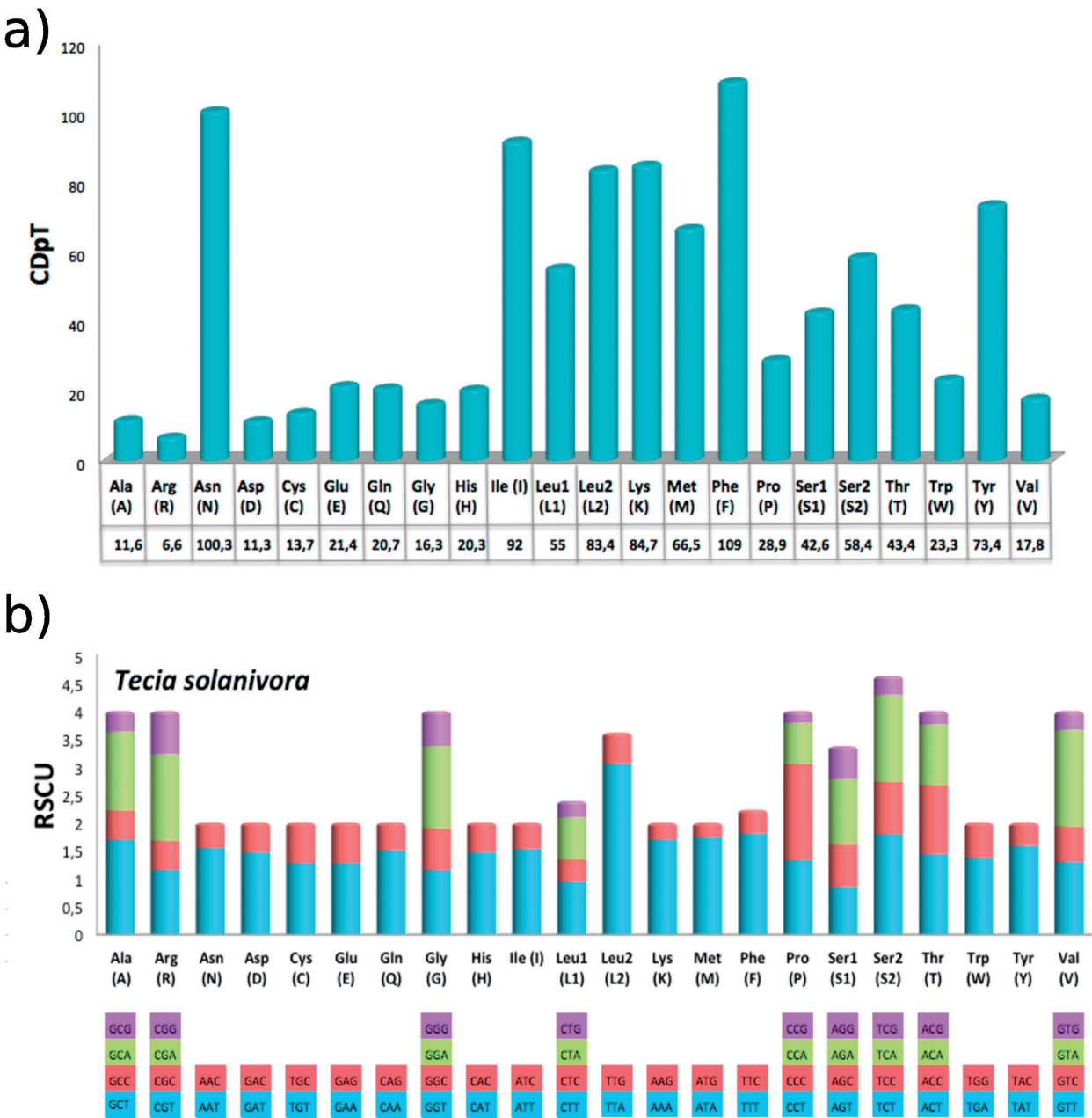


Figure 4. Codon distribution and relative synonymous codon usage (RSCU) in *T. solanivora* mitogenome. (a) Codon distribution and (b) RSCU. Codon families are provided on the X axis and the RSCU on the Y axis. This mitogenome presents all possible codon families existing in Lepidoptera.

3.5. Transfer RNA and ribosomal RNA genes

It was found that *T. solanivora* contains a typical set of 22 tRNAs with a high A + T bias, accounting for 80.7% of the tRNAs, slightly positive AT-skew (0.004) and a clearly positive GC-skew (0.161). These results suggest that tRNAs exhibit a higher inclination for nitrogen bases A and G than for T and C. Similar results were reported by [46] in *E. pyretorum* (AT-skew = 0.039 and GC-skew = 0.174) and [38] in *A. melete* (AT-skew = 0.034 and GC-skew = 0.142). Among the tRNA genes, 14 are coded on the H-strand and eight on the L-strand with lengths ranged

between 65 and 71 bp, and these genes exhibited positive AT-skew (0.047) as well as in almost all lepidopteran mitogenomes. However, the rearrangement of certain tRNAs were found translocated in the *T. solanivora* mitogenome compared with out-groups, which are described in (Figure 3).

Similar to other mitochondrial sequences from insect species, there were two rRNAs in *T. solanivora* with a total length of 2099 bp and an AT content of 83.7% (Table 2). The large ribosomal gene (rRNA-Large), located between tRNA-Leu1 and tRNA-Val, has a length of 1329 bp, whereas the small gene (rRNA-Small), located between tRNA-Val and the A + T-rich region, has a length of 770 bp (Table 1). These rRNA lengths were within the range of values reported for other Lepidoptera, as their values range between 1314 bp in *Euploea mulciber* (Nymphalidae) [47] and 1330 bp in *Coreana raphaelis* (Lycaenidae) [48]. For the rRNA-Large and rRNA-Small, the length ranges from 739 bp for *Protantigius superans* (Lycaenidae) to 788 bp in (Nymphalidae) [49]. The rRNAs in *T. solanivora* have an A + T content of 83.7%, and similar values were reported for other Lepidoptera, including *C. cephalonica* (80.43%), *T. incertulas* (82.8%), and *Dichocrocis punctiferalis* (85.1%) [50].

3.6. Noncoding and overlapping regions

Most of the intergenic regions in this mitogenome were short (≤ 15 bp) and the total length of the noncoding regions in the mtDNA of *T. solanivora* was 199 bp. This region is composed by 21 intergenic spacer sequences, ranging from 1 to 54 bp and showed highly A + T-biased (88.8%) (Table 2). The intergenic spacers longer were denominated S1, S2, S3, and S4. Intergenic sequence S1 is commonly found in Lepidoptera mitogenome order between the tRNA-Gln and NAD2 genes and length ranges between 38 pb in *T. incertulas* and 88 bp in *Sasakia charonda* [50]. However, this region has not been identified in insects that belong to other orders [9].

This sequence (S1) could be considered as a mitogenome marker for Lepidoptera order, and it most likely originated from a partial NAD2 gene duplication [19]. Intergenic sequence S2 (23 bp) was found between rRNA-Large and tRNA-Val. Intergenic sequences S3 and S4 (17 bp) separate genes NAD6 and Cytb, and the tRNA-Ser2 and NAD1 genes, respectively. The latter sequence contains the “ATACTAA” motif, typically found in other lepidopterans [9, 23, 51]. This motif plays an apparent role as a recognition site for the protein implicated in mitochondrial transcription termination (mtTERM) [52]. Furthermore, this sequence has been recognized for being highly conserved, with a length ranging between 17 and 20 bp [23].

Furthermore, in the *T. solanivora* mitogenome, three principal overlap sequences were identified and were designated as OLS1, OLS2, and OLS3. OLS1 was found overlapping the tRNA-Phe and NAD5 genes. This sequence presents the greatest length, with a total of 17 bp. OLS2 was found between the tRNA-Trp and tRNA-Cys genes, with a total length of 8 bp and the 7-bp OLS3-overlapped genes ATP8 and ATP6, which are consistent with the same genes found in other lepidopterans, although they differ in length [37, 52]. In addition, an unusual overlap region (OLS1) was found between the tRNA-Phe and NAD5 genes; it is important to mention that this 17-bp region has not been reported before.

3.7. The A + T-rich region

The A + T-rich region is a noncoding region with 325 bp length located between rRNA-Small and tRNA-Met. The region contains 91.1% AT nucleotides, with negative AT- and GC-skew values (**Table 2**), meaning that it is biased for the nitrogen base thymine, as reported for the mitogenomes of other lepidopterans. One exception to this trend is *A. honmai*, which has a positive AT skew (0.028), indicating a bias for adenines [21]. The length of this region is variable in the other Lepidoptera, and it can be as long as 1270 bp, as reported in *Papilio bianor* (Papilionidae) [53].

This A + T region is a conserved structure commonly found in other Lepidoptera, which includes the “ATAGA” motif followed by a 17-bp poly-T stretch, just like in *T. solanivora* mitogenome. This motif is immediately followed by the tRNA-Met gene [35, 41, 54] and it seems it has an important role in the replication initiation in minor strand of mtDNA in addition to gene regulation [19, 30, 41]. Furthermore, eight microsatellite regions were identified within the mitogenome of *T. solanivora*, referred to as (TAA)₄, (AT)₈ and (TAT)₇. These were the most representative microsatellites found in the species, although the mononucleotide sequences, (T)₆ and (A)₁₀, were also identified [55]. These represent the relevant regions of this genome for future studies. Also, in most lepidopteran mitogenomes, the (AT)₈ microsatellite has been previously reported. This microsatellite is preceded by the “ATTTA” motif that is commonly found in other mitogenomes [9, 41]. In *S. funebris*, the same (AT)₈ microsatellite was identified as that found in *T. solanivora*. Nevertheless, most lepidopteran mitogenomes report (AT)_n, where n ranges from 7 to 12 [19, 23].

3.8. Phylogenetic relationships

To illustrate the phylogenetic relationship of *T. solanivora* (Lepidoptera: Gelechiidae) with other 16 Lepidoptera families, we used a concatenated set of PCGs of 72 other complete Lepidoptera mitogenomes obtained from GenBank with previous elimination of start and stop codons. The phylogenetic relationship among the eight Lepidoptera superfamilies was inferred using both Bayesian Inference and maximum likelihood methods, which produced similar topologies to previously analyzed phylogenies obtained for other lepidopterans. The results obtained with both methods produced similar and consistent topologies. Our results showed high support values for the majority of the nodes and thus the interrelationships are well-resolved within order Lepidoptera. We used *Aedes aegypti* (Diptera) and *Acrida cinerea* (Orthoptera) as out-groups, the phylogenetic trees revealed nine Lepidoptera clades. Species of the Papilionoidea, Noctuidae, Bombycidae, Geometridae, Pyralidae, Gelechiidae, Tortricidae, Yponomeutidae, and Hepialidae superfamilies cluster monophyletic groups, with strongly supported bootstrapped and posterior probabilities (100). All those results and analysis were published by authors of the present chapter in 2016 [7].

4. Other recent studies with mitogenomes of Lepidopteran considered crop pests

A search carried out on August 04, 2017 in Scopus database showed that after publishing the scientific paper made by authors from this chapter [7], scientists have published 62 other

studies on mitochondrial genomes of lepidopteran insects. Most of them were focused on understanding their composition, organization, motifs, and the inference of phylogenetic relationships between these organisms [41, 56–58]. However, recently, [59] reported besides of typifying the mitogenomes of *Mesophleps albilinella* and *Dichomeris ustalella* (Lepidoptera: Gelechiidae), the prediction of the secondary structures of the tRNAs [44, 60]. In this model, single polynucleotide chain form four or five arms to fold itself with each other, like a clover leaf, call them an acceptor arm, DHU or D arm, anti-codon arm, T ψ C arm, and variable arm [61], which play a role in proper folding of the tRNA into the L-shaped tertiary structure while modifications in or around the anti-codon loop contribute to the function of tRNAs in decoding [62]. Prediction of secondary structures of tRNAs has been using tRNAscan-SE 1.21, Mito/Chloroplast, invertebrate genetic code for the prediction of tRNA isotypes, and a cutoff of 1 score [63]. This method allowed to find and predict the secondary structure of 21 tRNAs in both species, except for the tRNA^{Ser} (AGN), which has a truncated DHU arm, with consideration given to the anti-codons [59]. Frequently, have been reported tRNA-like structures into the A + T-rich region in Lepidoptera [37, 49, 64], but it seems to be fake tRNAs of random secondary structures, owing to the reduced sequence complexity (>90% A + T) in this noncoding region [59], suggesting that are fake tRNAs of random secondary structures, owing to the reduced sequence complexity (>90% A + T) in this noncoding region [65].

On the other hand, we must highlight the importance of studying of insect pests mitogenomes, this allows to propose hypotheses related with the evolutionary origin of the different larval stages, which causes significant damage to crops during this state, and could predict which is the most adaptable state to each type of environment as for example in *Parapoynx crisonalis* moth (Lepidoptera: Crambidae) [66] in which its larvae are lacking tracheal gills because they are pest in aquatic crops [67]. This analysis also can be extrapolated to the study of moths that are also plagues only in larval state but in terrestrial crops such as *T. solanivora*, and in this way to be able to find the most viable way to control this type of pests.

5. Novel techniques for pest control using mtDNA

Pest species represent a major ongoing threat to global biodiversity, demanding effective management approaches are required that regulate pest numbers, while minimizing collateral damage to nontarget species. Species-specific pest controls have been developed in order to be long-lasting measures and effectives [68]. One of these methods is called the sterile insect technique (SIT), whereby sterile males are introduced into target populations, so that they could be produced continuously within the targeted populations for control, and thus reducing production of females when mating with them. However, the SIT generally requires continuous large-scale production and introduction of sterile evils to sustain population suppression [69].

At the level of maternally inherited mitochondrial DNA (mtDNA) has been identified naturally occurring mutations that cause male infertility. These mutations have little or no impact

on females, and hence are minimally or not selected against (i.e. are self-perpetuating in nature). Due to those kinds of mutations, have only been identified in some model systems such as mice and fruit flies, they are likely to be widespread in nature threatening small populations viability of endangered species. Currently, a novel variant of the SIT, is the recently proposed Trojan female technique (TFT), based on the use of naturally occurring mutations or induced by CRISPR-Cas9 (clustered, regularly interspaced, short palindromic repeats system) in the mtDNA [69]. The consortium aims to harness these mutations to develop a widely applicable capability for pest control, through the release of Trojan females carrying the mutations [68, 69].

With this technique, males that inherit these mutations will have fewer offspring than wild-type males, while females will remain normal (fertile). It is well known that mtDNA is generally maternally inherited, so this sex-bias in effects will reduce selection pressure against the TFT mutation. When females carrying the TFT mutation are released into a pest population, they could cause multi-generational population suppression. However, while promising well and scientific means to control pest populations or disease vectors, the release of genetically engineered animals raises into ethical issues and a debate is currently underway discussing safety and regulatory concerns [68, 69].

6. Conclusion

In this chapter, the complete mitochondrial genome of *T. solanivora* was presented as a model to understand how to characterize and study a mitogenome in insects. It was sequenced, analyzed, and compared with other lepidopteran insects. This mitogenome shares many features with those reported previously in Lepidoptera but exhibited several subtle differences in the codon distribution within the A + T region. The phylogenetic relationships of nine clades of the order Lepidoptera were developed using Bayesian and maximum likelihood inference, which provided well-supported results compared with other phylogenies based on both molecular and morphological traits. In addition, an update about other recent mitogenomes research done mainly over lepidopteran insects considered crop pests was made. On the other hand, it was shown a novel development based on induced mutations by CRISPR-Cas9 in the mitogenomes seeking applicable capability for pest control. The utility of all information presented in this chapter is to improve scientific databases and support the determination of lepidopteran population genetic studies in the future.

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References

- [1] Nutter FW, Teng PS, Royer MH. Terms and concepts for yield, crop loss, and disease thresholds. *Plant Disease*. 1993;**77**:211-215
- [2] Cerda R, Avelino J, Gary C, et al. Primary and secondary yield losses caused by pests and diseases: Assessment and modeling in coffee. *PLoS One*. 2017;**12**:e0169133
- [3] EC O. Crop losses to pests. *The Journal of Agricultural Science*. 2006;**144**:31
- [4] Kristensen NP, Scoble MJ, Karsholt O. Lepidoptera phylogeny and systematics: The state of inventorying moth and butterfly diversity. *Zootaxa*. 2007;**1668**:699-747
- [5] Ma H-F, Zheng X-X, Peng M-H, et al. Complete mitochondrial genome of the meadow moth, *Loxostege sticticalis* (Lepidoptera: Pyraloidea: Crambidae), compared to other *Pyraloidea* moths. *Journal of Asia-Pacific Entomology*. 2016;**19**:697-706
- [6] Cameron SL. How to sequence and annotate insect mitochondrial genomes for systematic and comparative genomics research. *Systematic Entomology*. 2014;**39**:400-411
- [7] Ramírez-Ríos V, Franco-Sierra ND, Alvarez JC, et al. Mitochondrial genome characterization of *Tecia solanivora* (Lepidoptera: Gelechiidae) and its phylogenetic relationship with other lepidopteran insects. *Gene*. 2016;**581**:107-116
- [8] Boore JL. Animal mitochondrial genomes. *Nucleic Acids Research*. 1999;**27**:1767-1780
- [9] Cameron SL, Whiting MF. 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*. 2008;**408**:112-123
- [10] Avise JC. *Molecular Markers, Natural History and Evolution*. New York: Chapman & Hall; 1994
- [11] Pulliandre N, Dupas S, Dangles O, Zeddám J, Capdevielle-Dulac C, Barbin K, Torres-Leguizamón M, Silvain J. Genetic bottleneck in invasive species: The potato tuber moth adds to the list. *Biological Invasions*. 2008;**10**:319-333
- [12] Torres-Leguizamón M, Dupas S, Dardon D, Gómez Y, Niño L, Carnero A, Padilla A, Merlin I, Fossoud A, Zeddám J, Lery X, Capdevielle-Dulac C, Dangles O, Silvain J. Inferring native range and invasion scenarios with mitochondrial DNA: The case of *T. solanivora* successive north-south step-wise introductions across Central and South America. *Biological Invasions*. 2011;**13**:1505-1519
- [13] Villanueva-Mejía DF, Ramírez-Ríos V, Arango-Lsaza RE, et al. Phylogeography of *Tecia solanivora* from Colombia based on cytochrome oxidase i and cytochrome b mitochondrial genes. *South West Entomology*. 2015;**40**:775-788
- [14] Zeddám JL, Orbe K, Lery X, Dangles O, Dupas S, Silvain J. An isometric virus of the potato tuber moth *Tecia solanivora* (Povolny) (Lepidoptera: Gelechiidae) has a tri-segmented RNA genome. *Journal of Invertebrate Pathology*. 2008;**99**:204-211

- [15] Villanueva D, Saldamando CI. *Tecia solanivora*, Povolny (Lepidoptera: Gelechiidae): una revisión sobre su origen, dispersión y estrategias de control biológico. *Ing y Cienc.* 2013;**9**:197-214
- [16] Daniel ZR, Ewan B. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research.* 2008;**18**:821-829
- [17] Wyman Stacia K, Jansen RK, Bore JL. Automatic annotation of organellar genomes with DOGMA. *Bioinformatics.* 2004;**20**:3252-3255
- [18] Koichiro T, Daniel P, Nicholas P, Glen S, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution.* 2011:1-9
- [19] Cao S-S, Du Y-Z. Characterization of the complete mitochondrial genome of *Chilo auricilius* and comparison with three other rice stem borers. *Gene.* 2014;**548**:270-276
- [20] YP W, Li J, Zhao JL, TJ S, Luo AR, Fan RJ, Chen MC, Wu CS, Zhu C. The complete mitochondrial genome of the rice moth, *Corcyra cephalonica*. *Journal of Insect Science.* 2012;**12**:1536-2442
- [21] Eun-Seung L, Sik SK, Moo-Sang K, Hyungjin P, Cho Soowon KC-B. The mitochondrial genome of the smaller tea tortrix *Adoxophyes honmai* (Lepidoptera: Tortricidae). *Gene.* 2006;**373**:52-57
- [22] Liu S, Xue D, Cheng R, Han H. The complete mitogenome of *Apocheima cinerarius* (Lepidoptera: Geometridae: Ennominae) and comparison with that of other lepidopteran insects. *Gene.* 2014;**547**:136-144
- [23] Lu H-F, Su T-J, Luo A-R, Zhu C-D, Wu C-S. Characterization of the complete mitochondrial genome of diurnal moth *Amata emma* (Butler) (Lepidoptera: Erebiidae) and its phylogenetic implications. *PLoS One.* 2013;**8**:1-14
- [24] Chen MM, Li Y, Chen M, Wang H, Li Q, Xia RX, Zeng CY, Li Y. Complete mitochondrial genome of the atlas moth, *Attacus atlas* (Lepidoptera: Saturniidae) and the phylogenetic relationship of Saturniidae species. *Gene.* 2014;**545**:95-101
- [25] Dai LS, Zhu BJ, Liu QN, Wei GQ, Liu CL. Characterization of the complete mitochondrial genome of *Bombyx mori* strain H9 (Lepidoptera: Bombycidae). *Gene.* 2013;**519**:326-334
- [26] Hong MY, Lee EM, Jo YH, et al. Complete nucleotide sequence and organization of the mitogenome of the silk moth *Caligula boisduvalii* (Lepidoptera: Saturniidae) and comparison with other lepidopteran insects. *Gene.* 2008;**413**:49-57
- [27] Zhu BJ, Liu QN, Dai LS, Wang L, Sun Y, Lin KZ, Wei GQ, Liu CL. Characterization of the complete mitochondrial genome of *Diaphania pyloalis* (Lepidoptera: Pyralidae). *Gene.* 2013;**527**:283-291
- [28] Coates BS, Sumerford DV, Hellmich RL, Lewis LC. Partial mitochondrial genome sequences of *Ostrinia nubilalis* and *Ostrinia furnicalis*. *International Journal of Biological Sciences.* 2005;**1**:13-18

- [29] Kim JS, Park JS, Kim MJ, Kang PD, Kim SG, Jin BR, Han YS, Kim I. Complete nucleotide sequence and organization of the mitochondrial genome of eri-silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae). *Journal of Asia-Pacific Entomology*. 2012;**15**:162-173
- [30] Wang JP, Cao TW, Xuan SB, Wang H, Zhang M, bo Ma E. The complete mitochondrial genome of *Sasakia funebris* (Leech) (Lepidoptera: Nymphalidae) and comparison with other Apaturinae insects. *Gene*. 2013;**526**:336-343
- [31] Behura SK, Lobo NF, Haas B, deBruyn B, Lovin DD, Shumway MF, Puiu D, Romero-Severson J, Nene V, Severson D. Complete sequences of mitochondria genomes of *Aedes aegypti* and *Culex quinquefasciatus* and comparative analysis of mitochondrial DNA fragments inserted in the nuclear genomes. *Insect Biochemistry and Molecular Biology*. 2011;**41**:770-777
- [32] Nian L, Yuan H. Complete mitochondrial genome sequence of *Acrida cinerea* (Acrididae: Orthoptera) and comparative analysis of mitochondrial genomes in orthoptera. *Comparative and Functional Genomics*. 2010:1-16
- [33] Yukuhiro K, Sezutsu H, Itoh M, Shimizu K, Banno Y. 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*. *Molecular Biology and Evolution*. 2002;**19**:385-1389
- [34] Yang L, Wei ZJ, Hong GY, Jiang ST, Wen L. The complete nucleotide sequence of the mitochondrial genome of *Phthonandria atrilineata* (Lepidoptera: Geometridae). *Molecular Biology Reports*. 2009;**36**:1441-1449
- [35] Paola S, Simonato Mauro BA, Negrisolo E. The complete mitochondrial genome of the bag-shelter moth *Ochrogaster lunifer* (Lepidoptera, Notodontidae). *BMC Genomics*. 2008;**9**:331
- [36] Pan M, Yu Q, Xia Y, et al. Characterization of mitochondrial genome of Chinese wild mulberry silkworm, *Bombyx mandarina* (Lepidoptera: Bombycidae). *Science in China. Series C, Life Sciences*. 2008;**51**:693-701
- [37] Kim MI, Baek JY, Kim MJ, Jeong HC, Kim KG, Bae CH, Han YS, Jin BR, Kim I. Complete nucleotide sequence and organization of the mitogenome of the red-spotted apollo butterfly, *Parnassius bremeri* (Lepidoptera: Papilionidae) and comparison with other lepidopteran insects. *Molecules and Cells*. 2009;**28**:347-363
- [38] Hong G, Jiang S, Yu M, Yang Y, Li F, Xue F, Wei Z. The complete nucleotide sequence of the mitochondrial genome of the cabbage butterfly, *Artogeia melete* (Lepidoptera: Pieridae). *Acta Biochimica et Biophysica Sinica Shanghai*. 2009;**41**:446-455
- [39] Liu Y, Li Y, Pan M, Dai F, Zhu X, Lu C, Xiang Z. The complete mitochondrial genome of the Chinese oak silkmoth, *Antheraea pernyi* (Lepidoptera: Saturniidae). *Acta Biochimica et Biophysica Sinica Shanghai*. 2008;**40**:693-703
- [40] Fenn JD, Cameron SL, Whiting M. The complete mitochondrial genome sequence of the *Mormon cricket* (*Anabrus simplex*: Tettigoniidae: Orthoptera) and an analysis of control region variability. *Insect Molecular Biology*. 2007;**16**:239-252

- [41] Kim MJ, Wang AR, Park JS, et al. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperiidae) and phylogenetic reconstruction of Lepidoptera. 2014;**549**: 97-112.
- [42] Margam VM, Coates BS, Hellmich RL, Agunbiade T, Seufferheld MJ, et al. Mitochondrial genome sequence and expression profiling for the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae). PLoS One;**6**. Epub ahead of print 2011. DOI: 10.1371/journal.pone.0016444
- [43] Stewart JB, Beckenbach A. Characterization of mature mitochondrial transcripts in *Drosophila*, and the implications for the tRNA punctuation model in arthropods. Gene. 2009;**445**:49-57
- [44] Clary DO, Wolstenholme DR. Genes for cytochrome c oxidase subunit I, URF2, and three tRNAs in *Drosophila* mitochondrial DNA. Nucleic Acids Research. 1983;**11**:6859-6872
- [45] Ojala D, Montoya J, Attardi G. tRNA punctuation model of RNA processing in human mitochondria. Nature. 1981;**290**:470-474
- [46] Jiang ST, Hong GY, Yu M, Li N, Yang Y, Liu YQ, Wei ZJ. Characterization of the complete mitochondrial genome of the giant silkworm moth, *Eriogyna pyretorum* (Lepidoptera: Saturniidae). International Journal of Biological Sciences. 2009;**5**:351-365
- [47] Hao J, Sun M, Shi Q, Sun X, Shao L, Yang Q. Complete mitogenomes of *Euploea mulciber* (Nymphalidae: Danainae) and *libythea celtis* (Nymphalidae: Libytheinae) and their phylogenetic implications. ISRN Genomics. 2013;**14**
- [48] Kim I, Lee EM, Seol KY, et al. The mitochondrial genome of the Korean hairstreak, *Coreana raphaelis* (Lepidoptera: Lycaenidae). Insect Molecular Biology. 2006;**15**:217-225
- [49] Kim MJ, Jun J, Kim I. Complete mitochondrial genome of the mulberry white caterpillar *Rondotia menciaana* (Lepidoptera: Bombycidae). Mitochondrial DNA. 2014;**25**:1-3
- [50] Y-P W, Zhao J-L, T-J S, et al. The complete mitochondrial genome of *Leucoptera malifoliella* Costa (Lepidoptera: Lyonetiidae). DNA and Cell Biology. 2012;**31**:1508-1522
- [51] Yong-Qiang C, Ma C, Ji-Yue C, Yang DR. The complete mitochondrial genomes of two ghost moths, *Thitarodes renzhiensis* and *Thitarodes yunnanensis*: The ancestral gene arrangement in Lepidoptera. BMC Genomics. 2012;**13**:276
- [52] Taanman J. The mitochondrial genome: Structure, transcription. Biochimica et Biophysica Acta (BBA): Bioenergetics. 1999;**1410**:103-123
- [53] Hou LX, Ying S, Yang XW, Yu Z, Li HM, Qin X. The complete mitochondrial genome of *Papilio bianor* (Lepidoptera: Papilionidae), and its phylogenetic position within Papilionidae. Mitochondrial DNA. Epub ahead of print 2014. DOI: 10.3109/19401736.2013.873923
- [54] Zhao JL, Zhang YY, Luo AR, Jiang GF, Cameron SL, Zhu C. The complete mitochondrial genome of *Spilonota lechriaspis* Meyrick (Lepidoptera: Tortricidae). Molecular Biology Reports. 2011;**38**:3757-3764

- [55] Van Oppen MJH, Hislop NR, Hagerman PJ, Miller D. Gene content and organization in a segment of the mitochondrial genome of the scleractinian coral *Acropora tenuis*: Major differences in gene order within the anthozoan subclass Zoantharia. *Molecular Biology and Evolution*. 1999;**16**:1812-1815
- [56] Zhu X-Y, Xin Z-Z, Wang Y, et al. The complete mitochondrial genome of *Clostera anachoreta* (Lepidoptera: Notodontidae) and phylogenetic implications for *Noctuoidea* species. *Genomics*. 2017;**109**:221-226
- [57] Jeong SY, Kim MJ, Kim S-S, et al. Complete mitochondrial genome of the endangered Lycaenid butterfly *Shijimiaeoides divina* (Lepidoptera: Lycaenidae). *Mitochondrial DNA Part A*. 2017;**28**:242-243
- [58] Zou Z, Min Q, Cheng S, et al. The complete mitochondrial genome of *Thitarodes sejilaensis* (Lepidoptera: Hepialidae), a host insect of *Ophiocordyceps sinensis* and its implication in taxonomic revision of *Hepialus* adopted in China. *Gene*. 2017;**601**:44-55
- [59] Park JS, Kim MJ, Jeong SY, et al. Complete mitochondrial genomes of two gelechioids, *Mesophleps albilinella* and *Dichomeris ustalella* (Lepidoptera: Gelechiidae), with a description of gene rearrangement in Lepidoptera. *Current Genetics*. 2016;**62**:809-826
- [60] Chen M, Tian L-L, Shi Q-H, et al. Complete mitogenome of the Lesser Purple Emperor *Apatura ilia* (Lepidoptera: Nymphalidae: Apaturinae) and comparison with other nymphalid butterflies. *Dongwuxue Yanjiu*. 2012;**33**:191-201
- [61] Castro LR, Austin a D, Dowton M. Contrasting rates of mitochondrial molecular evolution in parasitic Diptera and Hymenoptera. *Molecular Biology and Evolution*. 2002;**19**:1100-1113
- [62] Dyubankova N, Sochacka E, Kraszewska K, et al. Contribution of dihydrouridine in folding of the D-arm in tRNA. *Organic & Biomolecular Chemistry*. 2015;**13**:4960-4966
- [63] Lowe TM, Eddy SR. TRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research*. 1996;**25**:955-964
- [64] Kim MJ, Jeong HC, Kim SR, et al. Complete mitochondrial genome of the nerippe fritillary butterfly, *Argynnis nerippe* (Lepidoptera: Nymphalidae). *Mitochondrial DNA*. 2011;**22**:86-88
- [65] Cameron SL, Lambkin CL, Barker SC, et al. A mitochondrial genome phylogeny of Diptera: Whole genome sequence data accurately resolve relationships over broad timescales with high precision. *Systematic Entomology*. 2007;**32**:40-59
- [66] Walker F. 'Pyralides', List of the Specimens of Lepidopterous Insects in the Collection of the British Museum London: Printed by order of the Trustees 1859. p. 509-1036
- [67] Chen Q, Chen Z-S, X-S G, et al. The complete mitogenome of *Parapoynx crisonalis* (Walker, 1859) (Lepidoptera: Crambidae), with phylogenetic relationships amongst three acen-tropine larval forms. *Aquatic Insects*. 2017;**38**:79-91

- [68] Dowling DK, Tompkins DM, Gemmell NJ. The trojan female technique for pest control: A candidate mitochondrial mutation confers low male fertility across diverse nuclear backgrounds in *Drosophila melanogaster*. *Evolutionary Applications*. 2015;8:871-880
- [69] Wolff JN, Tompkins DM, Gemmell NJ, et al. Mitonuclear interactions, mtDNA-mediated thermal plasticity, and implications for the Trojan Female Technique for pest control. *Scientific Reports*. 2016;6:30016