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Characterization of the complete mitochondrial genome of *Diaphania pyloalis* (Lepidoptera: Pyralididae)

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ABSTRACT

The complete mitochondrial genome (mitogenome) of *Diaphania pyloalis* (Lepidoptera: Pyralididae) was determined to be 15,298 bp and has the typical gene organization of mitogenomes from lepidopteran insects. It consists of 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes and an A + T-rich region. The A + T content of this mitogenome is 80.83% and the AT skew is slightly positive. All PCGs are initiated by ATN codons, except for cytochrome c oxidase subunit 1 (*cox1*) gene which is initiated by CGA. Only the *cox2* gene has an incomplete stop codon consisting of just a T. All the tRNA genes display a typical clover-leaf structure of mitochondrial tRNA. The A + T-rich region of the mitogenome is 332 bp in length, including several common features found in lepidopteran mitogenomes. Phylogenetic analysis showed that the *D. pyloalis* is close to Pyralididae.

1. Introduction

The mitochondrial genome (mitogenome) forms a unit of genetic information and evolves independently from the nuclear genome. Mitochondrial DNA has been widely used for diverse evolutionary studies (Wolstenholme, 1992). Insect mitogenome DNA (mtDNA) is a circular molecule containing 13 protein coding genes (PCGs), subunits 6 and 8 of the ATPase (*atp6* and *atp8*), cytochrome B (*cob*), cytochrome c oxidase subunits 1–3 (*cox1–cox3*), NADH dehydrogenase subunits 1–6 and 4L (*nad1-6* and *nad4L*), two ribosomal RNA genes encoding the small and large subunit rRNAs (*rrnL* and *rrnS*), 22 transfer RNA (tRNA) genes and a A + T-rich region (Boore, 1999; Moritz et al., 1987; Wolstenholme, 1992).

Up to now, only a few lepidopteran mitogenomes are available from species in the Bombycoidea, Geometroidea, Noctuoidea, Pyraloidea, Papilionoidea and Tortricoidea (Table 1). The complete or partial mitogenomes of six species of Pyraloidea had been sequenced: *Cnaphalocrocis medinalis* (Chai et al., 2012), *Chilo suppressalis* (Yin et al., 2011), *Ostrinia furnacalis* (Coates et al., 2005), *Ostrinia nubilalis* (Coates et al., 2005), *Maruca vitrata* (Margam et al., 2011) and *Diatraea saccharalis* (Li et al., 2011).

Diaphania pyloalis (Lepidoptera: Pyralididae), a member of well-known mulberry pests, is widely distributed in the main mulberry-growing regions and causes serious mulberry leaves yield losses. In the present study, the complete mitogenome of *D. pyloalis* was determined and the phylogenetic analyses to the selected species from Lepidoptera, Orthoptera and Diptera based on the mitogenome sequences were performed.

2. Materials and methods

2.1. DNA extraction

The larvae of *D. pyloalis* were collected from mulberry garden in Hefei, Anhui Province. All larvae were fed with fresh mulberry leaves under normal conditions. Total DNA from the larvae was extracted with the Genomic DNA Extraction Kit (AxyPrep, USA) and used for PCR amplification of the complete mitogenome.

2.2. PCR amplification and sequencing

Nine primer pairs were designed for PCR according to the conserved nucleotide sequences of known mitochondrial sequences from Lepidoptera insects (Chai et al., 2012; Coates et al., 2005; Li et al., 2011; Margam et al., 2011; Yin et al., 2011) (Table 2). The fragments were amplified using Aidlab 2 × PCR Master mix (Beijing, China) according to the manufacturer's instructions. The PCR was performed under the following conditions: 5 min at 94 °C, followed by 35 cycles of 35 s at 94 °C, 1–3 min at 50–60 °C, and 10 min at 72 °C. The PCR products were analyzed by agarose gel electrophoresis (1% w/v) and purified with a DNA gel extraction kit (AxyPrep, USA). The purified PCR products were ligated into the PMD-19 T vector

Abbreviations: PCR, polymerase chain reaction; ATPase, adenosine triphosphatase; NADH, nicotinamide adenine dinucleotide hydrogen.

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Table 1
The complete and near-complete mitochondrial genome of lepidoptera.

Species	Length (bp)	Accession number	References
Diaphania pyloalis	15, 298		This study
Cnaphalocrocis medinalis	15, 388	JN246082	Chai et al. (2012)
Chilo suppressalis	15, 465	HQ860290	Yin et al. (2011)
Ostrinia nubilalis	14, 535	NC_003367	Coates et al. (2005)
Ostrinia furnacalis	14, 536	AF467260	Coates et al. (2005)
Maruca vitrata	14, 054	HM751150	Margam et al. (2011)
Diatraea saccharalis	15, 490	FJ240227	Li et al. (2011)
Bombyx mori	15, 656	AB070264	Yukuhiro et al. (2002)
Japanese Bombyx mandarina	15, 928	NC_003395	Yukuhiro et al. (2002)
Chinese Bombyx mandarina	15, 682	AY301620	Pan et al. (2008)
Antheraea pernyi	15, 575	AY242996	Liu et al. (2008)
Antheraea yamamai	15, 338	EU726630	Kim et al. (2008)
Eriogyna pyretorum	15, 327	FJ685653	Jiang et al. (2009)
Samia cynthia ricini	15, 366	JN215366	Kim et al. (2012)
Actias selene	15, 236	JX186589	Liu et al. (2012)
Caligula boisduvalii	15, 360	NC_010613	Hong et al. (2008)
Manduca sexta	15, 516	EU286785	Cameron and Whiting, (2008)
Sphinx morio	15, 299	KC470083	Kim et al. (in press)
Hyphantria cunea	15, 481	GU592049	Liao et al. (2010)
Helicoverpa armigera	15, 347	GU188273	Yin et al. (2010)
Spodoptera exigua	15, 456	JX316220	Wu et al. (2013)
Cydia pomonella	15, 253	JX407107	Shi et al. (2013)
Ochrogaster lunifer	15, 593	AM946601	Salvato et al. (2008)
Phthonandria atrilineata	15, 499	EU569764	Yang et al. (2008)
Adoxophyes honmai	15, 680	DQ073916	Lee et al. (2006)
Grapholita molesta	15, 776	HQ116416	Gong et al. (2011)
Spilonota lechriaspis	15, 368	HM204705	Zhao et al. (2011)
Artogeia melete	15, 140	EU597124	Hong et al. (2009)
Coreana raphaelis	15, 314	NC_007976	Kim et al. (2006)
Acraea issoria	15, 245	GQ376195	Hu et al. (2010)
Fabriciana nerippe	15, 140	JF504707	Kim et al. (2011)
Kallima inachus	15, 183	JN857943	Qin et al. (2012)
Sasakia charonda	15, 233	JX119051	Wang et al. (2012)

(TaKaRa Co., Dalian, China) and sequenced at Invitrogen (Shanghai, China).

2.3. Sequence annotation

Sequence annotation was performed using the blast tools in NCBI web site (http://blast.ncbi.nlm.nih.gov/Blast) and DNAStar package (DNAStar Inc. Madison, USA). The nucleotide sequences of PCGs were translated with the invertebrate mitogenome genetic code and the PCGs were identified by sequence similarity with *C. suppressalis* (Yin et al., 2011). Identification of tRNA genes was verified using the tRNAscan-SE program. The potential stem-loop secondary structures within these tRNA gene sequences were calculated using the tRNAscan-SE Search Server (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Eddy, 1997). The secondary structures of tRNA genes that could not be predicted using the tRNAscan-SE were analyzed by comparison with the nucleotide sequences of other insect tRNA sequences (Chai et al., 2012; Coates et al., 2005; Li et al., 2011; Margam et al., 2011; Yin et al., 2011). Composition skewness was calculated according to the formulas: AT skew = [A − T]/[A + T]; GC skew = [G − C]/[G + C] (Junqueira et al., 2004). Tandem repeats in the A + T-rich region were predicted using the Tandem Repeats Finder program (http://tandem.bu.edu/trf/trf.html) (Benson, 1999). Alignments of PCGs from various lepidopteran mitogenomes were performed using Clustal X (Thompson et al., 1997).

Table 2
Primers used for amplification of the mitogenome of *D. pyloalis*.

Primer no.	Primer sequence (5′ → 3′)
F1	GCTTTTGGGCTCATACCTCA
R1	GATGAAATACCTGCAAGATGAAG
F2	TGGAGCAGGAACAGGATGAAC
R2	GAGACCADTACTTGCTTTCAG
F3	ATTTGTGGAGCTAATCATAG
R3	GGTCAGGGACTATAATCTAC
F4	TCGACCTGGAACTTTAGC
R4	GCAGCTATAGCCGCTCCTACT
F5	TAAAGCAGAAACAGGAGTAG
R5	ATTGCGATATTATTTCTTTTG
F6	ACATTCTTAGGTGGATTA
R6	GTTAAAGTGGCATTATCT
F7	GGAGCTTCTACATGAGCTTTTGG
R7	GTTTGCGACCTCGATGTTG
F8	GGTCCCTTACGAATTTGAATATATCCT
R8	AAACTAGGATTAGATACCCTATTAT
F9	CTCTACTTTGTTACGACTTATT
R9	TCTAGGCCAATTCAACAACC

2.4. Phylogenetic analysis

The mitogenomes of 32 lepidopteran species (including Bombycoidea, Geometroidea, Noctuoidea, Pyraloidea, Tortricoidea and Papilionoidea) were obtained from the GenBank database (Table 1) and used for phylogenetic analysis. The mitogenomes of *Locusta migratoria* (NC_001712), *Drosophila yakuba* (NC_001322) and *Anopheles gambiae* (NC_002084) were used as outgroups (Beard et al., 1993; Clary and Wolstenholme, 1985; Flook et al., 1995). Based on the concatenated set of amino acid sequences from the 13 PCGs, phylogenetic analysis was performed using neighbor-joining (NJ) method with the MEGA version 5.0 program (Tamura et al., 2011).

3. Results

3.1. Genome organization and base composition

As shown in Fig. 1, the mitogenome of *D. pyloalis* is 15,298 bp in length and contains typical organization of insect mitochondrial genomes, including 13 PCGs (*atp6* and *atp8*, *cob*, *cox1–3*, *nad1-6*, *nad4L*), 22 tRNA genes, two mitochondrial ribosomal RNAs (*rrnS* and *rrnL*) and a A + T-rich region. The order of genes and the orientation of the

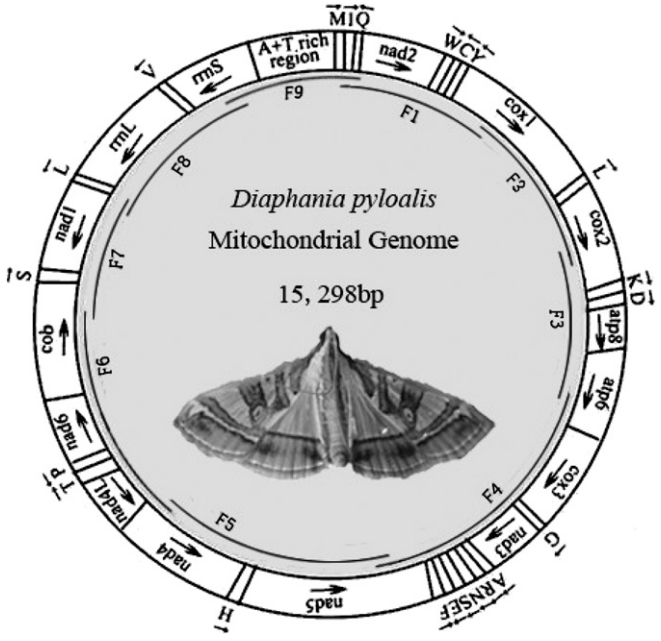

Fig. 1. Map of the mitogenome of *D. pyloalis*. tRNA genes are labeled according to the IUPAC-IUB. Single letter amino acids above the bar indicate coding sequence on major strand whereas below the bar indicates coding on minor strand. Anti-clockwise PCGs or rRNA genes are located on L strand and others are located on H strand.