Gene 577 (2016) 37–46

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Characterization of the complete mitochondrial genome of tea tussock moth, *Euproctis pseudoconspersa* (Lepidoptera: Lymantriidae) and its phylogenetic implications

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ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form 3 October 2015
Accepted 16 November 2015
Available online 27 November 2015

Keywords:
Mitochondrial genome
Euproctis pseudoconspersa
Noctuoidea
Phylogenetic relationship

ABSTRACT

In present work, we described the mitochondrial genome (mitogenome) of the tea tussock moth *Euproctis pseudoconspersa* (Lepidoptera: Lymantriidae). The complete mitogenome of *E. pseudoconspersa* is a circular genome 15,461 bp in size. It contains 37 genes and an A + T-rich region usually presented in lepidopteran mitogenomes, which genes share a lot of features with other known lepidopteran mitogenomes. Nucleotide composition of A + T in this mitogenome is 79.92%, and the AT skew is slightly positive. Both codon distribution and relative synonymous codon usage of the 13 protein-coding genes (PCGs) are consistent with those published lepidopteran sequences. All tRNA genes have typical cloverleaf secondary structures, except for the $tRNA^{Ser(AGN)}$, in which the dihydrouridine (DHU) arm is simplified down to a loop. The A + T-rich region of *E. pseudoconspersa* mitogenome possess the motif 'ATAGA' and poly-T stretch as the formerly identified conserved elements of Lepidoptera mitogenomes. The phylogenetic relationships were reconstructed by using maximum likelihood (ML) and Bayesian inference (BI) methods based on nucleotide sequences of 13 PCGs of 38 moths. The results were very consistent with the traditional relationships within Noctuoidea from morphological data, and showed that Lymantriidae is more closely related to Erebidae than to Noctuidae.

1. Introduction

Over the last decade, mitochondrial genomes (mitogenomes) have been studied increasingly because of the ease of recovering genetic information that may be useful for investigating molecular evolution as well as for phylogenetic and biogeographic studies (Shen et al., 2009; Timmermans et al., 2014; Ma et al., 2012). In addition, gene sequences, especially those of protein-coding genes, have been frequently utilized as an essential tool to estimate divergence times between different taxa (Pozzi et al., 2014). Because of the extensive use of its individual genes across a wide range of studies, mitogenomes had been great impacts on insect phylogenetics and molecular genetics. Recently, with the development of sequencing technologies, such as the second generation sequencing technology, mitogenomes can be sequenced reliably, cheaply and rapidly for almost all organisms (Cameron, 2014a). In parallel to these general developments, there has been a rapid increase in available mitochondrial genome data, for both animals in general and insects in particular (Cameron, 2014b).

Within the order Lepidoptera including butterflies and moths, there are more than 157,000 described species (Powell, 2003; Van Nieukerken et al., 2011). This order includes a number of biological model organisms. Many severe pest species, emphasizing that studies into both lepidopteran phylogeny and evolution are scientific and public interest. Among them, Noctuoidea is one of the largest superfamilies in the Lepidoptera, containing about 42,400 species worldwide (Van Nieukerken et al., 2011; Speidel and Naumann, 2004). So far, however, in such a big superfamily, only 16 species mtgenomes have been publicly available in GenBank (Table 1), only two species of which belong to the family Lymantriidae, i.e. *Gynaephora menyuanensis* (NC_020342) and *Lymantria dispar* (NC_012893).

Based on the previous studies, the monophyly of Noctuoidea seems well established (Miller, 1991; Kitching and Rawlins, 1998; Mitchell et al., 2000). However, within Noctuoidea (including Noctuidae, Notodontidae, Lymantriidae, Erebidae and Arctiidae), the family-level evolutionary relationships remain largely uncertain (Mitchell et al., 2000; Fibiger and Lafontaine, 2005). Zahiri et al. (2011) used one mitochondrial gene and seven nuclear genes regions to construct phylogenetic relationships in which proposed a newly robust phylogenetic

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Abbreviations: PCR, Polymerase chain reaction; *ATP6* and *ATP8*, Subunits 6 and 8 of the F_0 ATPase; *Cytb*, Cytochrome b; *COI–COIII*, Cytochrome c oxidase subunits 1–3; *Ile*, Isoleucine; *Leu*, Leucine; *ND1–ND6* and *ND4L*, NADH dehydrogenase subunit 1–6 and 4 L; *lrRNA* and *srRNA*, Large and small subunit of ribosomal RNA genes; $tRNA^{XXX}$, Genes encoding for transfer RNA molecules with corresponding amino acids denoted with a three-letter code and anticodon indicated in parentheses (XXX) when necessary; A + T-rich region, Adenine + thymine-rich region.

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http://dx.doi.org/10.1016/j.gene.2015.11.020
0378-1119/

Table 1
Taxonomy, GenBank accession numbers, and mitogenome sizes of 38 moths mitochondrial genomes used for the phylogenetic analysis. This table was created in November 2014, and sourced from GenBank databases.

Subfamily	Family	Species	Genome size (bp)	Acc. number	References
Bombycoidea	Bombycidae	*Bombyx mandarina*	15,928	NC_003395	Yukuhiro et al. (2002)
	Saturniidae	*Actias selene*	15,236	NC_018133	Liu et al. (2012)
		Antheraea pernyi	15,566	NC_004622	Liu et al. (2008)
		Eriogyna pyretorum	15,327	NC_012727	Jiang et al. (2009)
		Manduca sexta	15,516	NC_010266	Cameron and Whiting (2008)
	Sphingidae	*Sphinx morio*	15,299	NC_020780	Kim et al. (2013)
Hepialoidea	Hepialidae	*Ahamus yunnanensis*	15,816	NC_018095	Cao et al. (2012)
		Thitarodes renzhiensis	16,173	NC_018094	Cao et al. (2012)
		Thitarodes pui	15,064	NC_023530	Zou et al. (2011)
Noctuoidea	Noctuidae	*Agrotis ipsilon*	15,377	NC_022185	Wu et al. (2013a, 2013b, 2013c, 2013d, 2013e)
		Agrotis segetum	15,378	NC_022689	Wu et al. (2013a, 2013b, 2013c, 2013d, 2013e)
		Australothis rubrescens	15,382	NC_023791	Walsh (2014)
		Ctenoplusia agnata	15,261	NC_021410	Gong et al. (2013)
		Helicoverpa armigera	15,347	NC_014668	Yin et al. (2010)
		Mythimna separata	15,332	NC_023118	Unpublished
		Sesamia inferens	15,413	NC_015835	Unpublished
		Spodoptera exigua	15,365	NC_019622	Wu et al. (2013a, 2013b, 2013c, 2013d, 2013e)
		Spodoptera litura	15,388	NC_022676	Wan et al. (2013)
	Lymantriidae	*Gynaephora menyuanensis*	15,770	NC_020342	Yuan and Zhang (2013)
		Lymantria dispar	15,569	NC_012893	Unpublished
		Euproctis pseudoconspersa	15,461	KJ_716847	The present study
	Erebidae	*Hyphantria cunea*	15,481	NC_014058	Liao et al. (2010)
		Amata emma	15,463	NC_021416	Lu et al. (2013)
	Notodontidae	*Ochrogaster lunifer*	15,593	NC_011128	Salvato et al. (2008)
		Phalera flavescens	15,659	NC_016067	Hao (2012)
Pyraloidea	Crambidae	*Chilo suppressalis*	15,395	NC_015612	Chai et al. (2012)
		Cnaphalocrocis medinalis	15,388	NC_015985	Chai et al. (2012)
		Diatraea saccharalis	15,490	NC_013274	Li et al. (2011)
		Dichocrocis punctiferalis	15,355	NC_021389	Wu et al. (2013a, 2013b, 2013c, 2013d, 2013e)
		Elophila interruptalis	15,351	NC_021756	Park et al. (2013a, 2013b)
		Glyphodes quadrimaculalis	15,255	NC_022699	Park et al. (2013a, 2013b)
		Maruca vitrata	15,385	NC_024099	Margam et al. (2011)
		Paracymoriza distinctalis	15,354	NC_023471	Ye and You (2014)
Tortricoidea	Tortricidae	*Adoxophyes honmai*	15,680	NC_008141	Lee et al. (2006)
		Adoxophyes orana	15,343	NC_021396	Wu et al. (2013a, 2013b, 2013c, 2013d, 2013e)
		Cydia pomonella	15,253	NC_020003	Shi et al. (2013)
		Grapholita molesta	15,717	NC_014806	Son and Kim (2011)
		Spilonota lechriaspis	15,368	NC_014294	Zhao et al. (2011)

framework of Noctuoidea, but the lack of species of Lymantriidae. Therefore, a better understanding of Noctuoidea or Lymantriidae requires an expansion of taxon and genome samplings using more approaches and genetic markers for a strong phylogenetic signal.

The tea tussock moth, *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae), is a notorious pest feeding on the leaves of tea trees. In China and Japan, it has caused enormous loss in both quality and quantity of tea products. Moreover, the larvae have venomous spicules on their backs, and the spicules are harmful to human skin on contact (Ogata, 1958). Previous studies for the *E. pseudoconspersa* have mainly focused on the syntheses of sex pheromone (Ichikawa et al., 1995). Here, we described the *E. pseudoconspersa* mitochondrial genome as the first

Table 2
Regions and primers used in this paper.

Fragment	Region	Primer (J/N)	Primer sequence (J/N) 5′ → 3′
F1	*ND2*	N2-J[d]/N2-N-735[a]	TGATTTGGATGTTGAATTGG/CCAATAAATGGGGGTAATCCTCCTA
F2	*ND2-COI*	N2-C1-J[d]/N2-C1-N[d]	GCTTTAGGTGGATTAAATCAAAC/CAAATCCTAAAGCTCATAGAATTG
F3	*COI–COII*	C1-J-2167[a]/C1-N-3649[a]	TTGATTTTTCGGACATCCTGAAGT/CCGCAAATTTCTGAACATTGACCA
F4	*COI–COIII*	C1-C3-J[d]/C1-C3-N[d]	AGGTTTTATTGTTTGAGC/GCAGAAGATTTAGGGTCA
F5	*COIII*	C3-J-4317[a]/C3-N-4782[a]	TTATTTATTGTATCAGAAAT/TCTACGAAATGTCAATATCA
F6	*COIII-ND5*	C3-J-5407[d]/N5-N-7793[c]	GCTGCAGCTTGATATTGACA/AATCCTAATCCATCTCAACCT
F7	*ND5-ND4*	N5-J-7572[c]/N4-N-9153[c]	AAAAGGAATTTGAGCTCTTTTAGT/TGAGGTTATCAACCAGAGCG
F8	*ND4-ND4L*	N4-J-8941[c]/N-9629[c]	GAAACTGGGGCTTCAACATGAGC/GTTTGTGAAGGTTCTTTAGG
F9	*ND4L-Cytb*	N4L-708[d]/N-10991[d]	TATTATCTGAACCATTCAAAATATATTATG/AGCTAATAAAGATCCAAAATTTCATCAAT
F10	*Cytb*	CB-J-10933[a]/CB-N-11328[a]	TATGTACTACCATGAGGACAAATATC/GGCAAATAGGAAATATCATTC
F11	*Cytb*	N1-J-11046[a]/N1-N-11505[a]	TATGTACTACCATGGGGACAAATATC/ATTACTCCTCCTAGTTTATTAGGGAT
F12	*CytB-ND1*	CB-J-11335[c]/N1-N-12588[c]	CATATTCAACCCGAATGATA/AATCGAACTCCTTTTGATTTTGC
F13	*ND1*	N1-J-12197[a]/N1-N-12739[a]	TCTCCTTCACCTTCAGCAAAATC/ACAGCTTTTTTAGTGTTGATAGAACG
F14	*ND1-16S*	N1-J-12585[a]/16S-N-12945[a]	GGTCCCTTACGAATTTGAATATATCCT/GCGACCTCGATGTTGGATTAA
F15	*16S*	16S-J[d]/16S-N[d]	GGCTTACACCGGTTTGAACTCAGAT/GACTGTACAAAGGTAGCATAATCAT
F16	*16S-12S*	LR-J-13900[c]/N-12SR[d]	CTTGTGTATCAGAGTTTATTA/GTAAAAGTTCAAATAGCAAG
F17	*12S-ND2*	SR-N2-J[b]/N-N2[d]	TTTTAATAATAGGGTATCTAATCCTAGTTTTT/TGATGCTTCTGTAGAGGT

[a] Primers modified from Simon et al. (1994) up to this mtgenome.
[b] Primers modified from Lee et al. (2006) up to this mtgenome.
[c] Primers from Simon et al. (2006).
[d] Primers newly designed for this genome.