Journal of Asia-Pacific Entomology 17 (2014) 663–671

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

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

The complete mitochondrial genome of the endangered Apollo butterfly, *Parnassius apollo* (Lepidoptera: Papilionidae) and its comparison to other Papilionidae species

Yan-hong Chen [a], Dun-yuan Huang [a,b,c], Yun-liang Wang [a], Chao-dong Zhu [b], Jia-sheng Hao [a,*]

[a] Laboratory of Molecular Evolution and Biodiversity, College of Life Sciences, Anhui Normal University, Wuhu, PR China
[b] Institute of Zoology, Chinese Academy of Sciences, Beijing, PR China
[c] College of Forestry, Jiangxi Environmental Engineering Vocational College, Ganzhou, PR China

ARTICLE INFO

Article history:
Received 19 December 2013
Revised 8 April 2014
Accepted 6 June 2014
Available online 14 June 2014

Keywords:
Parnassiinae
Parnassius apollo
Mitochondrial genome
tRNA-like sequence
Intergenic spacer

ABSTRACT

The Apollo butterfly, *Parnassius apollo* is a representative species of the butterfly subfamily Parnassiinae. This charming species is one of the most endangered butterfly species in the world. In this study, we sequenced its complete mitochondrial genome (mitogenome), with the aim of accumulating genetic information for further studies of population genetics and mitogenome evolution in the Papilionidae. The 15,404-bp long mitogenome harbors a typical set of 37 genes and is the largest butterfly mitogenome determined, except for *Papilio maraho* (16,094 bp). Like many other sequenced lepidopteran species, one $tRNA^{Trp}$-like and one $tRNA^{Leu}$(UUR)-like sequences were detected in the AT-rich region. A total of 164 bp of non-coding sequences are dispersed in 14 regions throughout the genome. The longest intergenic spacer (68 bp) is located between $tRNA^{Ser}$(AGN) and $tRNA^{Glu}$, and is the largest spacer at this location among Papilionidae species. This spacer may have resulted from an 8-fold repetition of a TTTCTTCT motif or a 4-fold repetition of a CTTTATTT motif.

Introduction

The Apollo butterfly, *Parnassius apollo* is distributed mainly in the mountainous areas of Europe and northwest of China. This beautiful and charming species is one of the largest *Parnassius* butterfly species, with a wingspan of about 70 mm (Carter, 2000). Its adults are decorated with large black eye-spots on the forewings and red eye-spots on the hind wings. The size and color of the striking eye-spots can change as their habitats vary, and a variety of subspecies have evolved in different areas. Mainly owing to the over-collection, habitat loss, the destruction of its host plant (*Sedum* and *Sempervivum* species) and climate change, the Apollo butterfly is now becoming endangered in some of its habitats (Collins and Morris, 1985). It consequently has been assessed as vulnerable (VU) in Appendix II in CITES (Collins and Morris, 1985; Still, 1996) and IUCN Red List (Gimenez, 1996), as well as listed as Grade-II protected by the Chinese government.

The typical insect mitochondrial genome (mitogenome) has a circular structure about 15–16 kb long, and contains 37 genes, including 13 protein coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosome RNA (rRNA) genes and a non-coding region (i.e., the control region or the AT-rich region) (Wolstenholme, 1992; Boore, 1999). In view of its maternal inheritance and strict orthology, the lack of recombination and an accelerated evolutionary rate compared to nuclear genome, mitogenome has become popular in comparative and evolutionary genomics, molecular evolution, phylogenetics, and population genetics (Nardi et al., 2003, 2005; Simon et al., 2006; Cameron, 2014).

Up to the present, 45 complete or nearly complete mitogenome sequences of true butterflies (superfamily: Papilionoidea) have been reported including 8 species from the family Papilionidae. Within the subfamily Parnassiinae, there are two complete mitogenome sequences (Kim et al., 2009; Ji et al., 2012). Thus, more mitogenomic data of representative species, especially of endangered species, are very important for the studies of Papilionoidae phylogeny and ecology.

In this study, the complete mitogenome sequence of *P. apollo* was determined using the long PCR and the conserved primer walking methods, and the sequence was analyzed to determine gene arrangement, nucleotide composition and secondary

* Corresponding author at: No.1, EastBeijing Road, Wuhu, Anhui Province, China.

http://dx.doi.org/10.1016/j.aspen.2014.06.002
1226-8615/

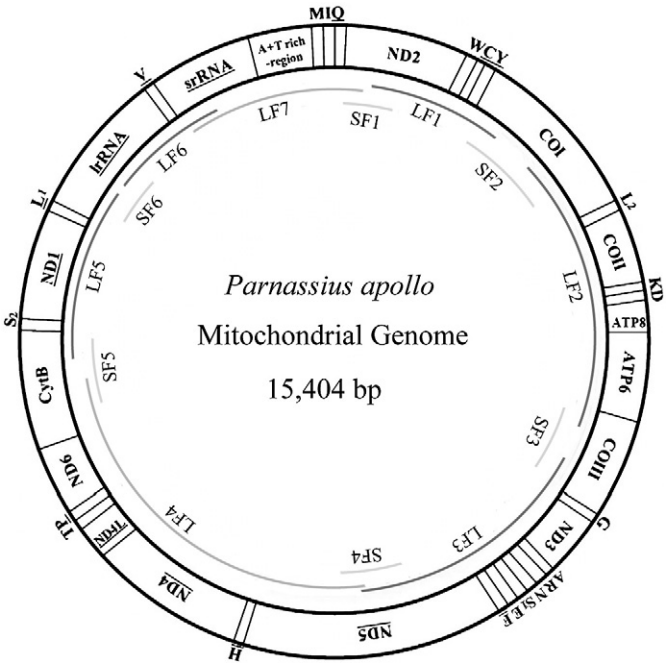

Fig. 1. Circular map of the mitochondrial genome of *Parnassius apollo*. The abbreviations for the genes are as follows: COI, COII, and COIII refer to the cytochrome oxidase subunits, CytB refers to cytochrome B, ATP6 and ATP8 refer to subunits 6 and 8 of F_0 ATPase, and ND1-6 refers to components of NADH dehydrogenase. tRNAs are indicated by the IUPAC-IUB single letter amino acid codes, while L1, L2, S1, and S2 denote $tRNA^{Leu}$(CUN), $tRNA^{Leu}$(UUR), $tRNA^{Ser}$(AGN) and $tRNA^{Ser}$(UCN), respectively. Gene names that are not underlined indicate transcription on the majority strand whereas underlines indicate transcription on the minority strand. The *P. apollo* mitogenome was sequenced by 6 short fragments (SF1-SF6) and 7 long fragments (LF1-LF7) as templates, shown as single lines within a circle.

Table 1
List of primers used to amplify and sequence the mitogenome of *Parnassius apollo*.

Fragment name	Primer name	Direction[e]	Sequence(5′–3′)	Nucleotide position[f]	Mismatch[g]	Annealing temperature(°C)
Short fragments						
SF1	ND2-F[a]	F	CGTTCATTTCTATTTCAGC	298–316	2	47.3
	ND2-R[a]	R	ACACCACCTATTGTTCCTA	718–736	1	
SF2	k698[b]	F	TACAATTTATCGCCTAAACTTCAGCC	1699–1721	3	46.9
	k807[b]	R	TGAAAATGAGCTACAACATAATA	2548–2570	0	
SF3	COIII-F[a]	F	ATCTCAATGATGACGAGAT	4859–4878	1	46.8
	COIII-R[a]	R	CAAATCCAAAATGGTGAGTA	5386–5405	3	
SF4	ND5-F[c]	F	AAAACTTCCAGAAAATAATCTC	6786–6807	5	46.5
	ND5-R[c]	R	TTGCTTTATCTACTTTAAGACA	7261–7282	1	
SF5	REVCB2H[d]	F	TGAGGACAAATATCATTTTGAGGW	10895–10918	1	45.0
	REVCBJ[d]	R	ACTGGTCGAGCTCCAATTCATGT	11498–11520	3	
SF6	lrRNA-F[a]	F	TACGCTGTCATCCCTAA	12976–12992	1	47.5
	lrRNA-R[a]	R	AAGTCTAATCTGCCCAC	13,337–13,353	0	
Long fragments						
LF1	ND2-COI-F[a]	F	CCCTTTCATTTCTGATTCC	564–582	1	51.1
	ND2-COI-R[a]	R	ACTGTTCGTCCTGTTCCT	1803–1820	2	
LF2	COI-COIII-F[a]	F	TCACAAGAAAGTGGAAAA	2212–2229	0	47.2
	COI-COIII-R[a]	R	TCTCTCATCGTAAGCCT	4929–4945	3	
LF3	COIII-ND5-F[a]	F	GCTGATAGTATTTATGGTTC	5263–5282	0	47.7
	COIII-ND5-R[a]	R	TTGTATGTGCTGGAGTT	7152–7168	1	
LF4	ND5-CytB-F[c]	F	AATTATACCAGCACATAT	7149–7166	3	47.1
	ND5-CytB-R[c]	R	TTATCGACTGCAAATC	10992–11007	2	
LF5	CytB-lrRNA-F[a]	F	TCCTGCTAACCCTTTAGTCA	11263–11282	3	49.2
	CytB-lrRNA-R[a]	R	GAGTATTTTGTTGGGGT	13082–13098	0	
LF6	lrRNA-srRNA-F[a]	F	CTGGGGTCTTCTCGTCT	13158–13174	2	51.6
	lrRNA-srRNA-R[a]	R	GCAATAAGTTGGCGGTA	14495–14511	3	
LF7	srRNA-ND2-F[a]	F	GAAACACTTTCCAGTACCT	14139–14157	0	49.7
	srRNA-ND2-R[a]	R	CTAAACCAATTCAACATCC	330–348	1	

[a] Primers newly designed for this genome.
[b] Primers from Caterino and Sperling (1999).
[c] Primers from Zhao et al. (2013).
[d] Primers from Simmon and Weller (2001).
[e] F and R, forward and reverse direction of transcription.
[f] Nucleotide positions are with respective to *Parnassius apollo* mitogenome.
[g] Mismatches are with respective to *P. apollo* mitogenome.