Complete mitogenome of the Lesser Purple Emperor *Apatura ilia* (Lepidoptera: Nymphalidae: Apaturinae) and comparison with other nymphalid butterflies

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Abstract: The complete mitochondrial genome of *Apatura ilia* (GenBank accession no. JF437925) was determined as a circular DNA molecule of 15 242 bp, with common genes of 13 putative proteins, 2 rRNAs, and 22 tRNAs and of the same gene arrangement as in other sequenced lepidopterans. All protein-coding genes had the typical start codon ATN, except for the COI’s using CGA as its start codon as previously demonstrated in other lepidopteran species. The comparison of the nucleotide sequences of the *A. ilia* mitogenome with ten other Nymphalidae species showed nearly identical gene orientation and arrangement, with only a few alterations in non-coding fragments. The nucleotide composition and codon frequency all fell into the range estimated for the order Lepidoptera. The *A. ilia* mitochondrial genome had the canonical set of 22 tRNA genes folded in the typical cloverleaf structure, with an unique exception of tRNA^Ser(AGN). The mitochondrial genes from *A. ilia* were overlapped in a total of 33 bp at 9 locations, as well as interleaved with a total of 155 bp intergenic spacers, spread over 12 regions with the size ranging from 1 to 49 bp. Furthermore, the spacer between ND6 and Cyt b harbored a microsatellite-like repeat (TA)23 not found in other completely sequenced nymphalid genomes. The 403 bp AT-rich region harbored two conserved motifs (ATAGA, ATTTA), a 21 bp polyT stretch, a 10 bp poly-A region, along with two microsatellite-like repeats ((TA)10 and (TA)7), as detected in other nymphalid butterflies.

Key words: Mitochondrial genome; Lepidoptera; Nymphalidae; *Apatura ilia*

柳紫闪蛱蝶线粒体基因组全序列及与相关蛱蝶类的比较分析

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摘要: 该文对柳紫闪蛱蝶 *Apatura ilia* (鳞翅目: 蛱蝶科) 的线粒体基因组全序列进行了测定，同时结合其它已知蛱蝶类的相应序列进行了比较分析。结果显示：柳紫闪蛱蝶的线粒体基因组 (GenBank accession no.: JF437925) 是一个 15 242 bp 的环状 DNA 分子，包含 13 个蛋白质编码基因、2 个 rRNA 基因和 22 个 tRNA 基因。13 个蛋白编码基因中，除了 COI 基因的起始密码子是 CGA 外，其余 12 个蛋白编码基因都具有标准的 ATN 起始密码子; 柳紫闪蛱蝶与其它已测的 10 种蛱蝶在基因定位和排列顺序方面几乎相同，只是在非编码序列上存在细微的差异，其核苷酸的构成及密码子使用频率都处于鳞翅目昆虫的范围之内。22 个的 tRNA 基因中，除了 tRNA^Ser(AGN) 缺少 DHU 臂，其余的 tRNA 基因都显示为典型的三叶草结构。基因组共存在 9 处基因间重叠区 (总长度为 33 bp) 以及 12 个基因间隔区 (总长度为 49 bp)，在 ND6 和 Cyt b 间的间隔区中还发现有 (TA)23 微卫星结构。与其他蛱蝶类相似，403 bp 的 AT 富集区包含有 ATAGA, ATTTA 二个保守模态 (一个 21 bp 的 poly-T, 一个 10 bp 的 poly-A)，以及二个微卫星的重复结构 ((TA)10 和 (TA)7)。

关键词：线粒体基因组; 嵛蝶目; 蛱蝶科; 柳紫闪蛱蝶
Present butterfly taxonomy classifies the Lesser Purple Emperor *Apatura ilia* into the subfamily Apaturinae (Lepidoptera: Nymphalidae) (Chou, 1998). This butterfly species was widely distributed in the Palaearctic Europe and Asia. Its name comes from the butterfly’s bright violet reflection under sunshine. In its lifecycle, larvae feed on the leaves of trees including trembling poplar, poplar, aspen and willow, whereas the adults mainly feed on tree sap and animal dejecta (Chou, 2000). Due to habitat destruction and environmental degradation, *A. ilia* has been designated as endangered or under priority protection in some countries or regions, including Belgium (Li & Fu, 2000).

The mitochondrial genome of insects is a circular and double-stranded molecule of approximately 14-20 kb in size with highly conserved exon arrangement covering a set of 37 genes, namely 13 PCGs, 22 tRNA genes, and 2 rRNA (srRNA (12S) and lrRNA (16S)) genes (Boore, 1999; Taanman, 1999). A mitogenome features smaller sizes, faster evolutionary rates, higher conservative gene content, maternal inheritance and little recombination (Brown, 1983; Avise, 1994), compared to the nucleic genome. Thus it has been commonly used for taxonomic and phylogenetic studies in many animal groups. The availability of complete mitogenome data of more species remarkably increases the accuracy and efficiency of a variety of research areas, such as molecular phylogenetics, phylogeography and taxonomy.

The complete mitochondrial genomes of nearly 240 insect species are available, of which only 10 are of Papilionoidae, despite its high biodiversity of 17 500 species (Robbins, 1982). The Nymphalidae is the largest butterfly family, and relationships with other butterfly groups remain unclear. It is necessary to integrate mitogenome data in the reconstruction of Nymphalidae phylogenies. In this study we sequenced the entire mitogenome of the nymphalid *A. ilia*, a representative butterfly species of the subfamily Apaturinae and compared its nucleotide organization to those of other representative nymphalid butterfly species. Our aim is to provide important molecular data to clarify the phylogenetic relationship between *A. ilia* and other nymphalid butterflies.

1 Materials and Methods

1.1 Sample collection and DNA extraction

Adult individuals of *A. ilia* were collected from Mount Yandangshan, Zhejiang, China in August 2008. Samples were quickly preserved in 100% ethanol and at -20 °C until DNA extraction. Total genomic DNA was isolated from a single frozen butterfly using the proteinase-K-SiO2 as follows (Hao et al, 2005). The thorax muscle around 5 mm³ was removed into a 10 mL Eppendorf tube, washed twice with ddH₂O, and soaked for 2~3 h. Incubation was done with 500 μL DNA solution (5 mmol/L NaCl, 0.5% SDS, 15 mmol/L EDTA, 10 mmol/L Tris-HCl, pH 7.6) and 40 μL proteinase-K (20 mg/mL). The muscle was then bathed at 55 °C for 10~12 h and centrifuged at 4 000 rpm for 2 min. Liquid supernatant was transferred to a new 10 mL Eppendorf tube with 500 μL 8 mol/L GuSCN and 40 μL 50% clean glass liquid mixture, bathed at 37 °C for 1~2 h, shocked every ten min and centrifuged at 4 000 rpm for 1 min. The supernatant was removed and sediments were cleaned twice with 75% alcohol, and once with acetone. The sample was dried thoroughly in a vacuum dryer at 45 °C prior to the addition of 60 μL TE (10 mmol/L Tris-Cl, 1mmol/L EDTA, pH 8.0). The solution was later bathed at 56 °C for 30 min, and centrifuged at increasing speed until 4 000 rpm for 1 min. The supernatant containing total genomic DNA was removed into a clean 1.5 mL Eppendorf tube and preserved at -20 °C for use.

1.2 Primer design, PCR amplification and DNA sequencing

The universal PCR primers for short fragment amplifications of the srRNA, COI and Cyt b genes were synthesized after Simon et al (1994) and Simons & Weller (2001). Long primers and certain short ones for some genes including COIII and ND5 were designed by the multiple sequence alignments of the complete mitochondrial genomes of all lepidopterans available (Tab. 1), using ClustalX 1.8 (Thompson et al, 1997) and Primer Premier 5.0 (Singh et al, 1998) softwares.

Long PCRs were performed using TaKaRa LA Taq polymerase with the cycling parameters: initial denaturation for 5 min at 95 °C, followed by 30 cycles of 95 °C for 50 sec, 47-61 °C for 50 sec, 68 °C for 2 min and 30 sec; and a final extension step of 68 °C for 10 min. The short fragments were amplified with TaKaRa Taq polymerase: initial denaturation for 5 min at 94 °C, followed by 35 cycles of 94 °C for 1 min, 45-53 °C for 1 min, 72 °C for 2 min; and a final extension step of 72 °C for 1 min.

> Supplementary information

The complete mitochondrial genome of *A. ilia* is available from GenBank accession number KF800237.
for 10 min. The PCR products were detected via electrophoresis in 1.2% agarose gel, purified using the 3S Spin PCR Product Purification Kit and sequenced directly with ABI–3730 automatic DNA sequencer. Mitogenome sequence data have been deposited into GenBank under the accession number JF437925.

1.3 Sequence analysis

All genes and the AT-rich region of the *A. ilia* mitogenome ClustalX 1.8. The nucleotide sequences of protein-coding genes were translated according to the invertebrate mtDNA genetic code. Fifteen of the 22 tRNA genes were identified using the software tRNA Scan-SE 1.21 (Lowe & Eddy, 1997) and RNAstructure 4.3 (Mathews, 2006). The remaining 7 tRNA genes were drawn manually after comparison with known homologous regions of other lepidopteran insects. MEGA 5.0 software (Tamura et al, 2007) was used to analyze nucleotide composition and codon usage.

2 Results

2.1 Gennome organization

The complete mitogenome of *A. ilia* is 15 242 bp in size. Similar to most insects, the mitogenome has a set of 37 genes: including 13 protein-coding, 22 tRNA and 2 rRNA genes. A large noncoding A+T-rich region was identified (Fig. 1). This region is of highly variable length in insects and is generally suggested to be the replication and transcription origin sites of the mtDNA double strands (Clayton, 1992). Furthermore the mitogenome of *A. ilia* was found to be highly similar to most sequenced lepidopterans in terms of gene order and orientation. Nine protein-coding genes were found in the major strand and the remaining 4 protein-coding genes in the minor strand along the mitogenome(Tab. 1). Besides those, the mitogenome of *A. ilia* has 9 overlapped sequences and 12 intergenic sequences.

2.2 Protein-coding genes, transfer RNA genes and ribosomal RNA genes

Thirteen protein-coding genes for 3 711 amino acids were identified in the mitochondrial genome of *A. ilia* (Tab. 2). The longest one is the COI gene with 1 533 bp and the shortest one is ATP8 with only 159 bp. Twelve protein-coding genes were initiated by conventional start codon ATN, while only the COI gene was tentatively designated to be CGA as the start codon. In *A. ilia*, nine protein-coding genes ended with TANs (7 with TAA, 2 with TAG), while four genes ended with a single T right ahead of tRNA genes (Tab. 1).

Twenty-two tRNA genes were found in the mitogenome (Fig. 2), all of the cloverleaf secondary structure except for tRNAser (AGN) which harbors a simple loop in the DHU arm. Twenty-two tRNA genes ranged from 62 bp for tRNAArg and tRNA Ser (AGN) to 71 bp for tRNAAsp and tRNAser (AGN) to 71 bp for tRNAAsp in length(Tab. 3).

As in other lepidopteran species, the *A. ilia* mitogenome was found to harbor two rRNA genes, srRNA (776 bp) and lrRNA (1,333 bp). They are located between tRNA Leu (CUA) and an A+T-rich region, separated by tRNA Val.

2.3 A+T-rich region, intergenic spacer and overlapping sequences

The AT-rich region of *A. ilia* was found to be 403 bp in length, located between srRNA and tRNA Met. It had the highest AT content (92.5%) across the whole mitogenome (Tab. 2), typical in Nymphalidae insects from 89.6% (*Melnitis leda* (Unpublished) to 96.3% (*Libythea celtis* (Unpublished)).In addition, the values of the AT skew and GC skew for this region reached to -0.07 and -0.14, respectively.

Thirteen intergenic spacer sequences were determined with a total length of 155 bp (Tab. 4). The longest two intergenic spacer sequences were both 49 bp long and located between tRNA Asp and ND2 and ND6 and Cyt b. The shortest one was only 1 bp in size. The
| Gene          | Direction | Nucleotide no. | Size | Anticodon     | Start codon | Stop codon |
|--------------|-----------|----------------|------|---------------|-------------|------------|
| tRNAMet      | F         | 1-68           | 68   | CAT32-34      |             |            |
| tRNAIle      | F         | 69-133         | 65   | GAT98-100     |             |            |
| tRNAGln      | R         | 131-199        | 69   | TTG235-237    |             |            |
| ND2          | F         | 249-1262       | 1014 | ATT           | TAA         |            |
| tRNA\textsuperscript{3p} | F         | 1261-1328      | 68   | TCA1292-1294  |             |            |
| tRNA\textsuperscript{3y} | R         | 1321-1387      | 67   | GCA1356-1358  |             |            |
| tRNA\textsuperscript{3r} | R         | 1388-1452      | 65   | GTA1419-1421  |             |            |
| COI          | F         | 1461-2993      | 1533 |               | CGA         | TAA        |
| tRNA\textsuperscript{3y}(UUR) | F         | 2989-3055      | 67   | TAA3019-3021  |             |            |
| COII         | F         | 3056-3731      | 676  | ATG           | T-tRNA      |            |
| tRNA\textsuperscript{3y} | F         | 3732-3802      | 71   | CTT3762-3764  |             |            |
| tRNA\textsuperscript{3y} | F         | 3803-3869      | 67   | GTC3834-3836  |             |            |
| ATPase8      | F         | 3870-4028      | 159  | ATT           | TAA         |            |
| ATPase6      | F         | 4022-4675      | 654  | ATG           | TAG         |            |
| COIII        | F         | 4684-5475      | 792  | ATA           | TAA         |            |
| tRNA\textsuperscript{3y} | F         | 5478-5542      | 65   | TCC5508-5510  |             |            |
| ND3          | F         | 5540-5894      | 355  | ATA           | T-tRNA      |            |
| tRNA\textsuperscript{4y} | F         | 5895-5960      | 66   | TGC5924-5926  |             |            |
| tRNA\textsuperscript{3y} | F         | 5960-6021      | 62   | TCG5987-5989  |             |            |
| tRNA\textsuperscript{4y} | F         | 6024-6088      | 65   | GTT6055-6057  |             |            |
| tRNA\textsuperscript{5y}(AGN) | F         | 6087-6148      | 62   | GCT6108-6110  |             |            |
| tRNA\textsuperscript{3y} | F         | 6151-6214      | 64   | TTC6179-6181  |             |            |
| tRNA\textsuperscript{3y} | R         | 6215-6278      | 64   | GAA6246-6248  |             |            |
| ND5          | R         | 6279-8010      | 1732 | ATT           | T-tRNA      |            |
| tRNA\textsuperscript{4y} | R         | 8011-8076      | 66   | GTG8042-8044  |             |            |
| ND4          | R         | 8077-9415      | 1339 | ATG           | T-tRNA      |            |
| ND4L         | R         | 9417-9701      | 285  | ATG           | TAG         |            |
| tRNA\textsuperscript{3y} | F         | 9714-9777      | 64   | TGT9744-9746  |             |            |
| tRNA\textsuperscript{3y} | R         | 9778-9841      | 64   | TGG9810-9812  |             |            |
| ND6          | F         | 9844-10371     | 528  | ATA           | TAA         |            |
| CytB         | F         | 10421-11572    | 1152 | ATG           | TAA         |            |
| tRNA\textsuperscript{5y}(UCN) | F         | 11571-11636    | 66   | GCT11601-11603|             |            |
| ND1          | R         | 11650-12591    | 942  | ATG           | TAA         |            |
| tRNA\textsuperscript{4y}(CUN) | R         | 12599-12666    | 68   | TAG12635-12637|             |            |
| LtRNA        | R         | 12667-13999    | 1333 |               |             |            |
| tRNA\textsuperscript{4x} | R         | 14000-14063    | 64   | TAC14032-14034|             |            |
| SrRNA        | R         | 14064-14839    | 776  |               |             |            |
| D-loop       |           | 14840-15242    | 403  |               |             |            |

\*tRNA abbreviations follow the IUPAC-IUB three-letter code.
Fig. 2. Predicted secondary cloverleaf structures for the 22 tRNA genes of *Apatura ilia*

The tRNAs are labeled after amino acid abbreviations. Nucleotide sequences from 5' to 3' are indicated for tRNA$^{\text{32}}$. Dashes (-) indicated Watson-Crick base-pairing, and centered asterisks (﹡) indicated G-U base-pairing. Arms of tRNAs (clockwise from top) are the amino acid acceptor (AA) arm, T$^\Psi$C(T) stem, the anticodon (AC) arm, and the dihydrouridine (DHU) arm.
**Tab. 2** Comparative characteristics of nymphalid mitogenomes

| Taxon               | Size (bp) | AT (%) | No. codons<sup>a</sup> | PCG<sup>b</sup> | AT (%) | Size | AT (%) | srRNA | AT (%) | D-loop | GenBank Accession No. | Reference               |
|---------------------|-----------|--------|--------------------------|-----------------|--------|------|--------|-------|--------|--------|----------------------|-------------------------|
| S. charonda         | 15244     | 79.9   | 3695                     | 78.2            | 1323   | 84.4 | 775    | 85.0  | 380    | 91.8   | NC014224             | GenBank                 |
| S. kurayamaensis    | 15222     | 79.9   | 3588                     | 78.3            | 1311   | 84.2 | 775    | 85.0  | 380    | 91.8   | NC014223             | GenBank                 |
| C. daudis           | 15267     | 80.4   | 3737                     | 78.8            | 1337   | 83.8 | 773    | 85.9  | 403    | 92.5   | HQ658143             | Xia et al, 2011         |
| A. hyperbius        | 15156     | 80.8   | 3718                     | 79.4            | 1330   | 84.5 | 778    | 85.4  | 349    | 95.4   | JF439070             | Wang et al, 2011        |
| A. ilia             | 15242     | 80.5   | 3711                     | 78.9            | 1333   | 86.0 | 776    | 84.9  | 403    | 92.5   | JF437925             | This study              |
| A. issoria          | 15245     | 79.7   | 3728                     | 78.0            | 1331   | 83.9 | 788    | 83.7  | 430    | 96.0   | GQ376195             | Hun et al, 2010         |
| H. autonoe          | 15489     | 79.1   | 3728                     | 76.8            | 1335   | 83.7 | 775    | 85.2  | 678    | 94.6   | GQ868707             | Kim et al, 2010         |
| M. leda             | 15122     | 79.8   | 3723                     | 79.0            | 1322   | 84.0 | 771    | 85.0  | 317    | 89.6   | JF905446             | Unpublished             |
| E. multiventer      | 15166     | 81.3   | 3725                     | 80.3            | 1314   | 84.6 | 776    | 85.3  | 399    | 93.5   | HQ378507             | Unpublished             |
| L. celtis           | 15164     | 81.2   | 3722                     | 79.9            | 1335   | 84.7 | 775    | 85.4  | 328    | 96.3   | HQ378508             | Unpublished             |
| A. metis            | 15236     | 80.5   | 3704                     | 78.9            | 1333   | 84.5 | 779    | 84.8  | 394    | 92.9   | NC015537             | GenBank                 |

<sup>a</sup>Termination codons were excluded in total codon count; <sup>b</sup>Protein-coding genes.

**Tab. 3** Sizes of each region of tRNAs in *Apatura ilia*

| Region                        | M | I | Q | W | C | Y | L<sup>1</sup> | K | D | G | A | R | N | S | E | F | H | T | P | S<sup>2</sup> | L | V |
|-------------------------------|---|---|---|---|---|---|-----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino-acylstem                | 68| 65| 69| 68| 67| 65| 67| 71| 67| 65| 66| 62| 65| 62| 64| 64| 66| 64| 64| 66| 68| 64|
| DHU stem                      | 4 | 3 | 4<sup>1</sup> | 4 | 4<sup>1</sup> | 3 | 3<sup>i</sup> | 3 | 4 | 4<sup>i</sup> | 4<sup>1</sup> | 3 | 3<sup>2</sup> | - | 4 | 4<sup>2</sup> | 4 | 4 | 4<sup>1</sup> | 4<sup>1</sup> | 3 | 4<sup>1</sup> | 4<sup>1</sup> |
| Anticodon stem                | 5 | 5 | 5<sup>i</sup> | 5 | 5 | 5<sup>i</sup> | 5 | 5 | 5 | 5 | 5 | 5<sup>i</sup> | 5 | 5 | 5 | 5 | 5 | 5<sup>i</sup> | 5 | 5 | 5 | 5 | 5 |
| TYPC stem                     | 6 | 6 | 5 | 6 | 4 | 8 | 7 | 6 | 5 | 4 | 4 | 4 | 4<sup>i</sup> | 5 | 3 | 3 | 4 | 5 | 5 | 5 | 5 | 5 | 6 | 4 |
| DHU loop                      | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Anticodon loop                | 4 | 5 | 4 | 4 | 4 | 4 | 4 | 5 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 7 | 4 | 4 | 5 | 4 | 4 | 4 | 3 | 4 |
| Variable loop                 | 5 | 4 | 7 | 7 | 10 | 4 | 5 | 9 | 6 | 5 | 4 | 5 | 4 | 3 | 8 | 4 | 6 | 6 | 4 | 3 | 4 | 8 | 5 |

Transfer RNA genes are labeled by one-letter symbols according to IUPAC-IUB single letter amino acid codes. L, L<sup>2</sup>, S and S<sup>2</sup> indicate tRNA<sub>Leu(CUN)</sub>, tRNA<sub>Leu(UUR)</sub>, tRNA<sub>Ser(AGN)</sub> and tRNA<sub>Ser(UCN)</sub>, respectively. Superscripts indicate number of mismatches in each region.

The mitogenome of *A. ilia* contains nine overlapping sequences ranging from 1 bp to 8 bp and totaling 33 bp in length.

### Discussion

#### 3.1 Gene organization and composition

The length of the complete mitogenome of *A. ilia* falls in the known range of the lepidopteran insects from 15 122 bp in *M. leda* to 16 094 bp in *Agehana marho* (*Papilio maraho*) (Wu et al, 2010). *A. ilia* demonstrated a common lepidopteran gene order tRNA<sub>Met</sub> followed by tRNA<sub>Ile</sub> and by tRNA<sub>Gln</sub>, different from those of other insect groups (tRNA<sub>Ile</sub> followed by tRNA<sub>Gln</sub> and by tRNA<sub>Met</sub>) (Tab. 1). Gene order was used to explore the presumed independent evolutionary lepidopteran lineages after divergence from their common ancestors (Boore et al, 1998).

The nucleotide composition of the *A. ilia* mitogenome showed considerable bias towards an A+T preference (80.5%) (Tab. 2), a common characteristic observed in insect mitochondrial genomes, ranging from 69.5% to 84.9 % (Crozier & Crozier, 1993; Dotson & Beard, 2001). It was noted that the content of base T (40.7%) was slightly higher than base A (39.8%), resulting in an AT skewness value of -0.012. The GC composition (19.5%) was correspondingly lower than AT (Tab. 2) and the GC skewness value was -0.21.

#### 3.2 Protein-coding genes

The putative start codons were found to be the same as in lepidopteran mtDNA (ATN codons: 3 with ATA, 6 with ATG, 3 with ATT) (Tab. 1), except that the COI gene has no uniform start codon (Lessinger et al, 2000; Yukuhiro et al, 2002). In general, lepidopteran insects were quite conservative in using CGA to initiate the COI gene, such as in *Eriogyna pyretorum* (Jiang et al, 2009), *Hyphantria cunea* (Liao et al, 2010) and *Adoxophyes honmai* (Lee et al, 2006). However, there are some
Tab. 4 Overlapping and intergenic spacer sequences of nymphalid mitogenomes

| Region                  | S.cha | S.kur | C.dau | A.hyp | A.ill | A.iss | H.aut | M.led | A.met | E.mul | L.cel |
|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| tRNA^Met^-tRNA^Ile      | -     | -     | -     | (O)2  | -     | -     | -     | -     | -     | -     | (I)9  |
| tRNA^Ile^-tRNA^Gln      | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  |
| tRNA^Gln^-ND2           | (I)27 | (O)15 | (O)2  | (O)2  | (O)2  | (I)4  | -     | (O)2  | (O)2  | (O)2  | (I)1  |
| tRNA^Gln^-tRNA^Cyto     | (O)8  | (O)8  | (O)8  | (O)8  | (O)8  | (O)8  | (O)8  | (O)8  | (O)8  | (O)8  | (O)8  |
| tRNA^Cyto^-tRNA^COI     | (O)5  | -     | -     | (O)1  | -     | (I)1  | -     | -     | -     | -     | (I)1  |
| tRNA^COI^-COI           | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  |
| tRNA^COI^-tRNA^Sep      | (O)1  | (O)1  | (O)20 | (O)1  | -     | (O)1  | (I)1  | (I)1  | -     | (O)1  | (O)1  |
| tRNA^Sep^-ATP8          | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| ATP8^-ATP6              | (O)7  | (O)7  | (O)7  | (O)7  | (O)7  | (O)7  | (O)7  | (O)7  | (O)7  | (O)7  | (O)7  |
| ATP6^-COII              | -     | -     | (O)1  | (O)1  | (I)8  | (O)1  | (O)1  | (O)1  | (O)1  | (O)1  | (O)1  |
| COII^-tRNA^Cyto         | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  |
| tRNA^Cyto^-ND3          | (O)3  | (O)3  | -     | (O)3  | -     | -     | -     | -     | -     | -     | -     |
| ND3^-tRNA^As                  | (O)2  | (O)2  | (I)22 | (I)8  | -     | (I)4  | -     | -     | (O)2  | (O)2  | (I)4  |
| tRNA^As^-tRNA^Sep         | (O)1  | (O)1  | -     | (I)2  | (O)1  | (I)2  | -     | (I)1  | (O)1  | (O)1  | (O)1  |
| tRNA^Sep^-tRNA^Cyto      | (I)7  | (O)10 | (O)2  | (O)2  | (O)2  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  | (O)3  |
| tRNA^Cyto^-tRNA^As       | (I)1  | (I)1  | (I)28 | (I)1  | (I)2  | (I)1  | (I)2  | -     | (I)8  | (I)1  | (I)1  |
| tRNA^As^-tRNA^8a         | (O)2  | (O)2  | (O)2  | (I)3  | -     | (O)2  | (O)2  | (O)2  | -     | (O)2  | (O)2  |
| tRNA^8a^-ND5             | (O)26 | (O)26 | (O)1  | (O)1  | -     | (O)1  | -     | (O)29 | -     | -     | -     |
| ND5^-tRNA^As             | (I)15 | -     | -     | (I)15 | -     | (I)8  | (O)3  | -     | (I)24 | (I)15  | -     |
| tRNA^As^-ND4             | (O)53 | (I)116| (O)1  | (O)1  | -     | (I)4  | -     | -     | -     | -     | -     |
| ND4^-ND4L               | (O)1  | (I)14 | (O)1  | (I)2  | (I)1  | -     | (O)1  | (I)23 | (I)1  | (O)1  | (O)1  |
| ND4L^-tRNA^8b            | (I)5  | (I)5  | (I)5  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  | (I)2  |
| tRNA^8b^-tRNA^Pro        | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| tRNA^Pro^-ND6            | (I)51 | (I)12 | (I)2  | (I)4  | (I)2  | (I)5  | (I)2  | (I)5  | (I)2  | (I)2  | (I)2  |
| ND6^-CytB               | (I)6  | (I)6  | (O)1  | (O)1  | (I)49 | (I)3  | (O)1  | (O)1  | (I)37 | (I)4  | (O)1  |
| CytB^-tRNA^As           | (I)2  | (I)355| -     | (O)2  | (O)2  | (I)8  | (I)8  | (O)2  | (O)2  | (I)2  | (I)2  |
| tRNA^As^-ND1            | (O)1  | (O)1  | (O)2  | (O)2  | (I)13 | (O)2  | (I)6  | (I)17 | (I)13 | (I)20 | (I)17 |
| ND1^-tRNA^As             | (I)10 | (I)1  | (I)1  | (I)3  | (I)7  | -     | (I)1  | (I)1  | (I)1  | (I)1  | (I)1  |
| tRNA^As^-trRNA           | -     | (I)1  | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| tRNA^Val^-srRNA          | (I)7  | (I)7  | -     | -     | -     | -     | -     | (O)1  | -     | -     | -     |
| tRNA^Val^-tRNA^Sr        | (I)1  | (I)1  | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Total nucleotides        | (I)36 | (I)607| (I)29 | (I)97 | (I)55 | (I)33 | (I)82 | (I)146| (I)151| (I)116| (I)196|

The 22 tRNA are denoted by three-letter symbol. Species are indicated by the initial letter of its genus and the initial three letters of its specific name (see the corresponding full name in Tab. 2). (O) Overlapping sequences; (I) Intergenic spacer. Empty column means neighboring genes abutting each other.

exceptions. A previous study using transcript information from the cDNA sequence showed that the start codon for the COI gene was TCG (Serine) in dipteran insects (Krzywinski et al, 2006). In addition, TTAAAG has been previously proposed to be the start codon for the COI gene in *Pieris rapae* (Mao et al, 2010), ATTAGC for *Papilio xuthus* (Feng et al, 2010), TAG for *Corean raphaelis* (Kim et al, 2006), and TG for *Acraea isorisa* (Hu et al, 2010) and *Calinaga davidis* (Xia et al, 2011).

In *A. ilia*, four genes ended with a single T right ahead of tRNA genes (Tab. 1). The single T residue could be completed into triplet codons by polyadenylation (Clary et al, 1985), and the tRNA secondary structure is functional to the precise cleavage of the mature protein-coding genes from the primary multicistronic transcripts (Ojala et al, 1980, 1981).
The AT bias of the protein-coding genes in *A. ilia* was prominent with AT content of 78.9%, the same as the average value of sequenced nymphalid mitogenomes (78.9%) (Tab. 2). Additionally, the PCG nucleotide frequency was T>A>G>C, displaying significant skews at AT (–0.15) and GC (0.02), both comparable to other sequenced lepidopterans (Liao et al, 2010). Examination of the concatenated 13 PCGs showed that the third codon position (91.7%) contained higher AT content than the first (74.4%) and second (70.9%) positions, and this case is also similar to other sequenced lepidopteran species like *Happrchia autonoe* (Kim et al, 2010). As for A+T content among 13 PCGs, ATP8 had the highest (93.9%) and COI has the lowest (72.4%) values (Tab. 2).

The *A. ilia* mitogenome also has an AT bias in codon usage. Among 3 711 codons there were 451 UUAs, 439 AUUs, 344 UUUs, 270 AUAs, 229 AAUs and 164 UAUs. These codons have much higher frequencies than others (Tab. 5).

### Tab. 5  Codon usage of PCGs in the *Apatura ilia* mitogenome

| Codon | Num. | RSCU | Codon | Num. | RSCU | Codon | Num. | RSCU | Codon | Num. | RSCU |
|-------|------|------|-------|------|------|-------|------|------|-------|------|------|
| UUU(F) | 344  | 1.81 | UCU(S) | 106  | 2.58 | UAU(Y) | 164  | 1.76 | UGU(C) | 30  | 1.76 |
| UUC(F) | 36   | 0.19 | UCC(S) | 9    | 0.22 | UAC(Y) | 22   | 0.24 | UGC(C) | 4   | 0.24 |
| UUA(L) | 451  | 5.03 | UCA(S) | 100  | 2.43 | UAA(*) | 0    | 0.00 | UGA(W) | 89  | 1.89 |
| UUG(L) | 25   | 0.28 | UCG(S) | 1    | 0.02 | UAG(*) | 0    | 0.00 | UGG(W) | 5   | 0.11 |
| CUU(L) | 38   | 0.42 | CUC(P) | 62   | 2.10 | CAU(H) | 56   | 1.65 | CGU(R) | 16  | 1.21 |
| CUC(L) | 4    | 0.04 | CCC(P) | 18   | 0.61 | CAC(H) | 12   | 0.35 | CGC(R) | 2   | 0.15 |
| CUA(L) | 20   | 0.22 | CCA(P) | 36   | 1.22 | CAA(Q) | 53   | 1.83 | CAG(R) | 30  | 2.26 |
| CUG(L) | 0    | 0.00 | CCG(P) | 2    | 0.07 | CAG(Q) | 5    | 0.17 | CGG(R) | 5   | 0.38 |
| AUU(I) | 439  | 1.88 | ACC(T) | 11   | 0.30 | AAC(N) | 24   | 0.19 | AGC(S) | 2   | 0.05 |
| AUC(I) | 27   | 0.12 | ACA(T) | 61   | 1.64 | AAA(K) | 93   | 1.79 | AGA(S) | 75  | 1.82 |
| AUA(M) | 270  | 1.81 | AC(A[T]) | 67   | 2.37 | GAA(E) | 66   | 1.81 | GGA(G) | 102 | 2.07 |
| AUG(M) | 29   | 0.19 | AC(G) | 0    | 0.00 | GAG(K) | 11   | 0.21 | GGG(G) | 30  | 0.61 |
| GUU(V) | 64   | 1.94 | GCU(A) | 67   | 2.37 | GAU(D) | 61   | 1.82 | GGU(G) | 53  | 1.08 |
| GUC(V) | 1    | 0.03 | GCC(A) | 8    | 0.28 | GAC(D) | 6    | 0.18 | GGC(G) | 12  | 0.24 |
| GUA(V) | 62   | 1.88 | GCA(A) | 37   | 1.31 | GAA(E) | 66   | 1.81 | GGA(G) | 102 | 2.07 |
| GUG(V) | 5    | 0.15 | GCG(A) | 1    | 0.04 | GAG(E) | 7    | 0.19 | GGG(G) | 30  | 0.61 |

A total of 3 711 codons are analysed, excluding the termination codons. Num: number of each codon. RSCU: relative synonymous codon usage. *: stop codons.

3.3 Transfer RNA genes and ribosomal RNA genes

The DHU arm of tRNA*ser* (AGN) has only a simple loop, common in most insects (Hong et al, 2008; Kim et al, 2006; Salvato et al, 2008; Wolstenholme, 1992). Similar to *Parnassius bremeri* (Kim et al, 2009), the tRNAs of *A. ilia* harbor 7 base pairs in amino-acyl stems, 5 base pairs in anticodon stems, and 7 base pairs in anticodon loop. However, the base pair numbers vary in other tRNA portions, especially within the TΨC loops (3-10 bp) (Tab. 3).

Among the 22 tRNA genes 32 mismatched base pairs were found, 10 on the amino acyl stem, 9 on the DHU stem, 1 on the TΨC stem and 9 on the anticodon stem. Twenty were between guanine and uracil, justifiable in terms of structural stableness (Topal & Fresco, 1976). Some unconventional mismatches were also observed, e.g. A-C (1), A-G (1), and U-U (8). Similar cases were also seen in other lepidopteran species. For example, *C. raphaelis* has 8 U-U mismatches in tRNAs; *A. issoria* exhibits a G-A and a C-U mismatches in the tRNA*ile*; *H. autonoe* contains a U-U and a A-C mismatches in tRNA*leu* (UUR), *H. cunea* has U-U mismatches in tRNA*ala*, tRNA*leu* (CUN), and tRNA*leu* (UUR) (Liao et al, 2010). These mismatches can be corrected through RNA-editing mechanisms that are well known for arthropod mtDNA (Lavrov et al, 2000).

As in other lepidopteran species, the *A. ilia* mitogenome was found to harbor two rRNA genes (776 bp srRNA and 1 333 bp lrRNA). The AT content of srRNA was 84.9%, similar to those of other lepidopteran insects (87.5% for *Phthionandria atrilineata* (Yang et al, 2009), 82.0% for *Ostrinia furnacalis* (Coates et al, 2005)) (Tab. 2). The AT content of lrRNA (85.0%) also fell into the range for other lepidopteran insects (85.1% for *P. atrilineata*, 81.4% for *Ostrinia Lunifer* (Salvato et al, 2008)) (Tab. 2).
3.4 Intergenic spacer and overlapping sequence

The spacer 1 of 49 bp was located between the tRNA^{Gln} and ND2 genes and this spacer had the same size to that in *O. furnicalis*, *Ostrinia nubilalis* (Coates et al, 2005), *B. mori* and *B. mandarina* (Yukihiro et al, 2002). It has been suggested that the spacer would remain invariant in length in some congeneric species (Cameron & Whiting, 2008), however, new findings showed divergence among *Apatura Metis* (unpublished, NC-015537) and *A. ilia* (Tab. 4). The comparison of this spacer with the neighboring ND2 gene showed 63% homology. Likewise, the cases for *H. autonoe* (74%), *P. bremeri* (70%), *P. atrilineata* (70%), *C. raphaelis* (62%), *A. melete* (70%) and *B. mori* (63%) all suggest that this spacer sequence originated from the ND2 gene (Kim et al, 2009).

Like spacer 1, spacer 2 was also 49 bp in length and found between ND6 and Cyt b. It is notable that a microsatellite-like repeat (TA)_{23} was identified within this region. This case is extremely rare among the known nymphaid mitogenomes (the other example is in *A. metis* which has (TA)_{12} in this region).

Spacer 3 was 13 bp and located between tRNA^{Ser} (CUN) and ND1. This spacer sequence held a 7-base motif ATACTAA. A similar motif has been identified in previous studies as a plausible conservancy in all Lepidoptera species sequenced so far (Kim et al, 2009; Cameron & Whiting, 2008; Liao et al, 2010; Salvato et al, 2008). It may be functionally essential in the recognition of the mtDNA TERM (the transcription termination peptide). Recent mitogenomic sequence data has demonstrated mixed results in Nymphalidae species. For example, in *H. autonoe* a 6-bp spacer (Kim et al, 2010) was located between tRNA^{Ser} (CUN) and ND1, and the 7-bp motif ATACTAA located within tRNA^{Ser} (CUN), whereas in *C. dauidis*, *A. hyperbius* and *A. issoris*, the two genes were overlapped for 1 or 2 bp and the motif was located at the 3’ end of the ND1 gene.

The 12-bp spacer 4 was located between ND4L and tRNA^{Thr}, being longer than those of other nymphaid species. All remaining spacers in *A. ilia* are less than 10 bp.

We also found two overlapping sequences which are conservative in Lepidoptera, one was 7 bp long and the other 8 bp. The 7-bp sequence was located between ATP8 and ATP6 as ATGATAA, and the 8-bp overlap was located between tRNA^{Gln} and tRNA^{Cys} as AAGCCTTA. These two sequences have also been detected in other lepidopteran insects, such as *P. bremeri*, *H. cunea* and *P. atrilineata*. Based on data of the presently sequenced lepidopteran insects, the two overlapping sequences were postulated to be conservative across lepidopteran insect taxa. Another 5-bp sequence was located between COI and tRNA^{Leu}. The remaining five overlapping sequences range from 1 to 3 in size. So far the 3-bp overlap between tRNA^{Asc} and tRNA^{Gln} has been found in all sequenced Nymphalidae species.

3.5 A+T-rich region

The AT-rich region functional in mtDNA replication and transcription (Taanman, 1999). The origin of the major-strand replication was studied in the AT-rich region in vertebrates (Tapper & Clayton, 1981), followed by the detection on both strands of mtDNA in *Drosophila* species (Clary & Wolstenholme, 1987; Fauron & Wolstenholmn, 1980). In recent years investigations were expended to the replication origin of the minor-strand in *Diptera*, Lepidoptera, Coleoptera and Orthoptera, and the results suggest that the replication origin site of mtDNA minor-strand in insects is located before the poly-T structure, which is standing at the 3’ end of the AT-rich region (Saito et al, 2005).

The AT-rich region of *A. ilia* has some common or similar structural features for lepidopteran insects. It harbors a 21-bp poly-T stretch located 18 bp upstream from srRNA and preceded by a motif ATAGA. The poly-T stretch and the motif composed the origin site for the minor-strand replication, recognizable as a structural signal by regulating proteins (Kim et al, 2009). Additionally, there are two microsatellite-like repeats of (TA)_{10} and (TA)_{7}, which are preceded by a conserved motif ATTTA and located upstream of (TA)_{10} repeat. At the 5’ end of this region there is a 14-bp poly-A structure, shortened to 10 bp by inserting a single T base. A similar case was found for other nymphaid butterfly species; for example, the poly-A of *A. issoris* mtDNA was inserted by a guanine. Functionally, the poly-A was assumed to be the replication origin location of the mtDNA major-strand because of its connection with tRNA^{Met} (Kim et al, 2009).

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**地图勘误说明**

地图事关国家安全、主权、民族尊严和中华民族的根本利益，历来受到国家的高度重视。依据《中华人民共和国地图编制出版管理条例》和《地图审核管理规定》的有关要求，中国地图全图中不得缺少香港特别行政区、澳门特别行政区、海南岛、台湾岛、钓鱼岛、南海诸岛、赤尾屿及海域国界线等。本刊 2011 年 32 卷第 181、569 和 578 页相应示意图的中国地图全图中缺少南海诸岛、钓鱼岛、赤尾屿及海域国界线等，特此勘误说明。希望此勘误能够引起作者和编者的足够重视。

本刊编辑部

2012 年 3 月 20 日
《猴、猿、人——思考人性的起源》新书简介

中山大学人类学系的张鹏著《猴、猿、人——思考人性的起源》一书已于2012年1月由中山大学出版社出版。书中全面讨论了人与猿猴的异同，尝试从灵长类进化的新视角探索人性起源，解释人类的共通性及其生物学本质，并为读者提供理解自身的新思维。目前国产灵长类的书籍不多，欢迎读者订阅！

《猴、猿、人——思考人性的起源》简介：
毫无疑问，人类是600余种灵长类中的一种。解剖学者说猿猴的手掌、指纹、大脑等身体结构与人类似。行为生态学者说猿猴的行为和复杂社会关系与人类似。人类学者说猿猴的工具使用技能和文化传播可能接近于人类的雏形阶段。心理学者说猿猴是地球上最聪明的动物。保护生物学学者说有猿猴生活的森林一定是最健康的森林，保护猿猴就是保护森林生态。医学工作者说猿猴的免疫系统接近于人，是克除人类疾病的最佳模式。分子生物学者说黑猩猩属与人类基因相似度高达98.7%，是与人类最近缘的物种。可以说，猿猴是一面多棱镜，从方方面面映射出人性起源的背景。

本书是国内第一本综合介绍灵长类知识的书籍。书中全面讨论了人与猿猴的异同，尝试从灵长类进化的新视角探索人性起源，解释人类的共通性及其生物学本质，并为读者提供理解自身的新思维。第一章介绍了人类的生物属性，第二章进一步详细介绍了近代灵长类学的诞生与发展过程；从第三章到第十章，分章节介绍了灵长类的起源与分类、生态、行为、社会、文化、智能、疾病与遗传、生命伦理与保护等各方面知识，第十一章总结了人类与猿猴的区别，以及解答如何界定人类长期未解的自身谜团。

《猴、猿、人——思考人性的起源》章节目录：
序一（日本京都大学渡边邦夫）
序二（中山大学麻国庆）
前言
第一章 人类的生物属性
第二章 灵长类学的诞生与发展
第三章 灵长类的起源与分类
第四章 灵长类的生态
第五章 灵长类的行为
第六章 灵长类的社会
第七章 灵长类的文化
第八章 灵长类的智能
第九章 灵长类的疾病与遗传
第十章 灵长类的生命与保护
第十一章 结语：人性的起源
参考文献
附录
后记

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友情链接：张鹏和渡边邦夫著《灵长类的社会进化》，2009年，中山大学出版社；可从网上购买，当当网http://product.dangdang.com/product.aspx?product_id=20727953。

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