



## The complete mitochondrial genomes of the Fenton's wood white, *Leptidea morsei*, and the lemon emigrant, *Catopsilia pomona*

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### Abstract

The complete mitochondrial genomes of *Leptidea morsei* Fenton (Lepidoptera: Pieridae: Dismorphiinae) and *Catopsilia pomona* (F.) (Lepidoptera: Pieridae: Coliadinae) were determined to be 15,122 and 15,142 bp in length, respectively, with that of *L. morsei* being the smallest among all known butterflies. Both mitogenomes contained 37 genes and an A+T-rich region, with the gene order identical to those of other butterflies, except for the presence of a tRNA-like insertion, *tRNA<sup>Leu</sup>* (UUR), in *C. pomona*. The nucleotide compositions of both genomes were higher in A and T (80.2% for *L. morsei* and 81.3% for *C. pomona*) than C and G; the A+T bias had a significant effect on the codon usage and the amino acid composition. The protein-coding genes utilized the standard mitochondrial start codon ATN, except the *COI* gene using CGA as the initiation codon, as reported in other butterflies. The intergenic spacer sequence between the *tRNA<sup>Ser</sup>* (UCN) and *ND1* genes contained the ATACTAA motif. The A+T-rich region harbored a poly-T stretch and a conserved ATAGA motif located at the end of the region. In addition, there was a triplicated 23 bp repeat and a microsatellite-like (TA)<sub>9</sub>(AT)<sub>3</sub> element in the A+T-rich region of the *L. morsei* mitogenome, while in *C. pomona*, there was a duplicated 24 bp repeat element and a microsatellite-like (TA)<sub>9</sub> element. The phylogenetic trees of the main butterfly lineages (Hesperiidae, Papilionidae, Pieridae, Nymphalidae, Lycaenidae, and Riodinidae) were reconstructed with maximum likelihood and Bayesian inference methods based on the 13 concatenated nucleotide sequences of protein-coding genes, and both trees showed that the Pieridae family is sister to Lycaenidae. Although this result contradicts the traditional morphologically based views, it agrees with other recent studies based on mitochondrial genomic data.

**Keywords:** mitochondrial genome, Pieridae, phylogenetic analysis

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## Introduction

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The animal mitochondrial genomes (mitogenomes) are usually circular molecules of 14–19 kb in size, containing 37 genes (including 13 protein-coding genes, two rRNA genes, and 22 tRNA genes) and a non-coding A+T-rich region that regulates the transcription and replication of the mitogenome (Clayton 1992). Due to its simple and compact structure, fast evolutionary rate, and maternal inheritance, it has been used frequently in the studies of population genetics, molecular evolution, phylogenetics, phylogeography, and evolutionary biology (Simon et al. 1994). In recent years, as the DNA sequencing technology has been progressing rapidly, more and more complete animal mitogenome sequences have been determined. To date, more than 240 complete or near-complete mitochondrial DNA sequences have been identified from insects, including 67 from lepidopterans. Of these, the available sequences are mainly from six superfamilies (Bombycoidea, Geometroidea, Papilionoidea, Noctuoidea, Tortricoidea, and Pyraloidea). In total, 33 of these lepidopteran mitogenomes are from butterflies (Table 1).

Pieridae is one of the largest families of Papilionoidea, containing 76 genera and approximately 1,100 species worldwide, mostly distributed in tropical Africa and Asia (Courtney 1986, Watt et al. 1996, Brunton 1998, Stavenga et al. 2004, Kemp et al. 2005). Their adults are generally of medium size and typically white, orange, and yellow in color (Chapman 1895). Taxonomically, they are currently divided into four subfamilies (Dismorphiinae, Pierinae, Coliadinae, and Pseudopontiinae). In addition, phylogenetically, they may stand as a key group to clarify the intra-familial butterfly relationships. For example, they were traditionally considered to be the sister to the Papilionidae (Ehrlich 1958,

Scott 1985). However, more and more evidence indicated that they were sister to the grouping of (Nymphalidae (Riodinidae, Lycaenidae)) (Kristensen 1976, de Jong et al. 1996, Weller et al. 1996, Ackery et al. 1999, Wahlberg et al. 2005) or sister to (Riodinidae + Lycaenidae) (Kim, M. J. et al. 2010, Chai et al. 2012). To our dissatisfaction, up to the present, only four mitogenomes of pierid species (*Artogeia melete* [Menetries], *Pieris rapae* [L.], *Delias hyparete* [L.], and *Aporia crataegi* [L.]) are available, and thus more pierid mitogenome data are needed to enrich the taxon sampling for use in phylogenetic studies.

The Fenton's wood white, *Leptidea morsei* Fenton, and the lemon emigrant, *Catopsilia pomona* (F.), are the two representative species of the family Pieridae. *Leptidea morsei* is distributed mainly throughout Europe, Siberia, Ussuri, Korea, northern China, and Japan. It is found occasionally in damp, grassy vegetation at the sunny edges of woods, as well as in grassy woodland. Its larvae feed on peas, and adults are seen twice per year from April to May and June to July. *Catopsilia pomona* is ubiquitously distributed from areas of south-east Asia (Sikkim, Malaysia, Philippines) to Australia. It is found often in secondary forests, along river courses, and even in the hot arid deserts throughout the year. Its colors are usually variable, chiefly lemon-yellow with an apical black margin (Rienks 1985).

In this study, we determined and analyzed the complete mitogenome sequences of these two pierid species and compared these sequences with those of other butterfly species available to clarify the phylogenetic relationships among the main butterfly lineages. The new sequence data will provide valuable information for the studies of lepidopteran comparative genomics, molecular evolution, and other relevant areas.

## Materials and Methods

### Sample collection

Adult individuals of *L. morsei* and *C. pomona* were collected from Shanxi and Hainan Provinces, China, in August 2008 and July 2009, respectively. After sample collection, the fresh materials were placed into 100% ethanol immediately for DNA fixation and stored at -20°C until used for genomic DNA extraction.

### DNA extraction and amplification by PCR

Total genomic DNA of *L. morsei* and *C. pomona* was extracted from the thoracic muscle of an adult individual by using the glass bead method after Hao et al. (2005). Insect universal primers were used for the amplification of the *COI*, *CytB*, *16S rRNA*, and *12S rRNA* genes (Simon et al. 1994). Primers for the *ND2*, *ND4*, *COIII*, and *ND5* amplification were designed via the alignment of the respective sequences from all the butterflies available by using Clustal X1.8 and Primer Premier 5.0 softwares (Thompson et al. 1997, Singh et al. 1998). Seven long fragments (*COI-COIII*, *COIII-ND5*, *ND5-CytB*, *CytB-16S*, *16S-12S*, *12S-ND2*, *ND2-COI*) were amplified via long PCR using Takara LA Taq™ (Takara Co., [www.takara-bio.com](http://www.takara-bio.com)). The long PCR conditions were as follows: an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 50 sec, annealing at 50–55°C (depending on primer pairs) for 50 sec, and elongation at 68°C for 150 sec during the first 15 cycles and then an additional 5 sec per cycle during the last 15 cycles, and a final extension at 68°C for 10 min. All PCR fragments were sequenced directly in both strands after purification with the QIA quick PCR Purification Kit (QIAGEN, [www.qiagen.com](http://www.qiagen.com)), except for the *12S-ND2* fragment of *L. morsei*, which was sequenced after cloning. All of

the long PCR fragments were sequenced by using the primer walking strategy.

### Sequence analysis

The raw sequences from the overlapping fragments were proofread and assembled in BioEdit version 7.0 (Hall 1999). Protein-coding genes, rRNA genes, and A+T-rich regions were determined via the alignment of the sequences by using Clustal X1.8 software (Thompson et al. 1997). The nucleotide sequences of the protein-coding genes were translated based on the invertebrate mtDNA genetic code. The tRNAs were identified by tRNAscan-SE software version 1.21 (Lowe and Eddy 1997). The putative tRNAs that could not be found by tRNAscan-SE were confirmed by sequence comparisons between the Pieridae and other butterfly tRNAs. Nucleotide composition and codon usage were calculated by using MEGA5.1 software (Kumar et al. 2004), and the tandem repeats in the A+T-rich regions were predicted by the Tandem Repeats Finder available online (<http://tandem.bu.edu/trf/trf.html>) (Benson et al. 1999). Sequence data were deposited in the GenBank database under the accession numbers JX274648 for *L. morsei* and JX274649 for *C. pomona*.

### Phylogenetic analysis

For the phylogenetic analysis, 13 concatenated nucleotide sequences of protein-coding genes of 33 available butterfly mitogenome sequences (two newly sequenced in this study and 31 extracted from GenBank, Table 1) were aligned by using Clustal X1.8 (Thompson 1997). The phylogenetic trees were then reconstructed with the maximum likelihood and Bayesian inference methods using the moth species *Adoxophyes honmai* Yasuda (Lepidoptera: Tortricidae) (GenBank accession number DQ073916) as the outgroup.

In the maximum likelihood and Bayesian inference analyses, the third position of all the codons was excluded, and the best fitting substitution model GTR + I + G (Lanave et al. 1984) was selected via a comparison of Akaike Information Criterion scores (Akaike 1974), calculated by using the Modeltest software version 3.7 (Posada and Crandal 1998). The maximum likelihood analyses were conducted in PAUP version 4.0b8 (Swofford 2002) under the following conditions: tree searching by TBR (tree bisection and reconnection) branch swapping (10 random-addition sequences); specifying the number of substitution rate categories as four; and using a BIONJ distance-based tree as the starting tree. The confidence values of each node of the maximum likelihood tree were evaluated via the bootstrapping test with 1,000 iterations. Bayesian analyses were performed by using the program MrBayes 3.1 (Huelsenbeck and Ronquist 2001). Two independent runs of four incrementally heated MCMC chains (one cold chain and three hot chains) were simultaneously run for one million generations in all datasets. Each set was sampled every 100 generations with a burn-in of 25%, and when the average standard deviation of split frequencies was less than 0.01, stationarity was considered to be reached. The confidence values of the Bayesian inference tree were presented as the Bayesian posterior probabilities in percentages.

## Results and Discussion

### General features

The complete mtDNA sequences of *L. morsei* and *C. pomona* were 15,122 and 15,142 bp in length, respectively, with that of *L. morsei* being the shortest among all known sequences of butterfly species (Table 2). Each genome was composed of the typical 13 protein-coding genes, 22 tRNA genes, two rRNA

genes, and one major non-coding A+T-rich region. The gene order was identical to that of other sequences of butterflies but different from that found in the ancestral insects with respect to the location of *tRNA<sup>Met</sup>*. That is to say, the *tRNA<sup>Met</sup>* was located between the control region and *tRNA<sup>Ile</sup>*, giving the derived control region (CR)-Met (M)-Ile (I)-Glu (Q) arrangement instead of that of the insect ground plan CR-I-Q-M (Fig. 1). The total sizes of protein-coding genes, rRNAs, and tRNAs were all well within the corresponding ranges of those found in other butterfly species (Table 2). The size proportions of coding genes to the whole genome of these two and four other pierid species were slightly higher than those of butterflies of other families (Table 3). By contrast, their non-coding sequences, including intergenic spacers and the A+T-rich region, were slightly shorter than those of other family taxa. The A+T-rich region of *C. pomona* was the shortest, whereas that of *Papilio maraho* Shiraki & Sonan (Lepidoptera: Papilionidae) was the longest (Table 4). The majority strand coding nine protein-coding genes and 14 tRNAs were 7,804 and 7,836 bp, respectively, whereas the minority strand coding four protein-coding genes, eight tRNAs, and two rRNA genes were 6,901 and 6,934 bp, respectively, for the two pierid species.

The nucleotide compositions of the two entire mitogenome sequences were biased significantly toward A and T (Table 5). These A+T contents were generally consistent with those of other butterfly mitogenomes, which ranged from 79.1% in *Eumenis autonoe* Esper (Lepidoptera: Nymphalidae) (Kim, M. J. et al. 2010) to 82.7% in *Coreana raphaelis* Oberthür (Lepidoptera: Lycaenidae) (Kim, I. et al. 2006). The base composition bias of an individual strand can be described by A+T skewness, calculated by  $(A\% - T\%) / (A\% + T\%)$ ,



and G+C skewness, calculated by  $(G\% - C\%) / (G\% + C\%)$ . The A+T and G+C skewness values in majority strands were calculated to be -0.122 and -0.121, respectively, for *L. morsei*, and -0.119 and -0.087, respectively, for *C. pomona*.

### Protein-coding genes

All the protein-coding genes in *L. morsei* and *C. pomona* started with a typical ATN codon, with the only exception represented by the CGA start codon of the *COI* gene. For *L. morsei*, three genes (*ND2*, *ND5*, *ND6*) started with ATT, one (*ATP8*) with ATC, two (*ND3*, *ND4*) with ATA, and six (*ATP6*, *ND1*, *COII*, *COIII*, *ND4L*, *CytB*) with ATG. In comparison with *L. morsei*, *ATP8*, *ND3*, and *ND6* in *C. pomona* possessed different start codons, namely ATT, ATT, and ATC, respectively. These start codons were well-conserved in the sequenced butterfly mitogenomes. For instance, the *ND2* gene usually used ATT as the start codon, whereas the *COII* and *ATP6* genes frequently used ATG as the start codon (Table 6).

Nine protein-coding genes were terminated with the standard stop codon TAA, whereas the *COI*, *COII*, *ND4*, and *ND5* genes used T as a truncated stop codon. The only difference in stop codons between the two pierid species was found in the *ND3* gene, that is, *L. morsei* used TAA instead of TAG, which appeared in *C. pomona*. Furthermore, incomplete stop codons were detected frequently in the *COI*, *COII*, and *ND5* genes in most insects, including all sequenced butterfly species (Table 6). Incomplete stop codons would produce functional stop codons after polycistronic transcript cleavage and polyadenylation (Ojala et al. 1981).

Previous studies reported that most lepidopterans used the codon CGA as the start codon for *COI* (Fig. 2). However, exceptions have

been reported; for example, TTG was proposed as the start codon in *Acraea issoria* Hübner (Lepidoptera: Nymphalidae) (Hu et al. 2010), *Caligula boisduvalii* Eversmann (Lepidoptera: Saturniidae) (Kim, I. et al. 2006), and *Fabriciana nerippe* Felder (Lepidoptera: Nymphalidae) (Kim, M. J. et al. 2011a); ATT in *A. crataegi* (Park et al. 2012) and *Ctenoptilum vasava* Moore (Lepidoptera: Hesperidae) (Hao et al. 2012); TTAG in *Bombyx mori* L. (Lepidoptera: Bombycidae) (Yukuhiro et al. 2002) and *C. raphaelis* (Hong, G. Y. et al. 2009); and ATTTAG in *Ostrinia nubilalis* Hübner and *Ostrinia furnacalis* Guenée (Lepidoptera: Crambidae) (Coates et al. 2005). In this study, a typical ATN initiator for *COI* in *L. morsei* and *C. pomona* was not detected at their starting sites. The putative ATT start codon is commonly located upstream of the *COI* gene and frequently followed immediately by the TAG or TAA stop codon; thus, the CGA, not ATT, probably acted as the start codon here as in most butterflies.

Excluding stop codons, the A+T contents of protein-coding genes in *L. morsei* and *C. pomona* were 79.16% and 80.02% (Table 5), respectively, and these values were similar to those detected in other butterflies, which ranged from 76.8% in *E. autonoe* (Kim, M. J. et al. 2010) to 81.5% in *C. raphaelis* (Kim, I. et al. 2006) (Table 2). When the first, second, and third codon positions were considered separately, the highest A+T contents were in the third positions for *L. morsei* and *C. pomona*. In addition, the highest T contents were detected in the second positions, and the lowest G contents in the third positions (Table 5).

Exclusive of the stop codon, 3,713 and 3,724 amino acids were encoded by the mitogenomes of *L. morsei* and *C. pomona*, respectively (Table 2). The amino acid numbers were well within the size range of 3,586

in *Sasakia charonda kuriyamaensis* Shirozu (Lepidoptera: Nymphalidae) (Hakozaki et al., unpublished, GenBank accession number NC\_014223.1) to 3,740 in *Abisara fylloides* Moore (Lepidoptera: Riodinidae) (Shi et al. unpublished, College of Life Sciences, Anhui Normal University, China) detected in other butterflies. Among the amino acids, UUU (Phe), UUA (Leu), AUU (Ile), AUA (Met), and AAU (Asn) were the most frequently used codons in *L. morsei* and *C. pomona* (Table 7), and similar codons were found in other butterfly species, such as *C. vasava* (Hao et al. 2012), *A. crataegi* (Park et al. 2012), and *Sericanus montela* Gray (Lepidoptera: Papilionidae) (Ji et al. 2012).

### Transfer RNAs and ribosomal RNAs

There were 22 tRNA genes (two each for serine and leucine, and one for each of the other amino acids) identified within the two pierid mitogenomes. An additional tRNA-like sequence (*tRNA<sup>Leu</sup>* [UUR]) located within the *16S rRNA* gene was detected in the mitogenome of *C. pomona*. The 22 tRNA genes ranging in size from 60 to 71 bp were interspersed throughout the two whole mitogenomes. The total sizes of the *L. morsei* and *C. pomona* tRNAs were 1,416 and 1,446 bp, respectively, with 80.68 and 81.05% A+T contents, respectively. All tRNAs could be folded into the typical clover leaf secondary structure, whereas *tRNA<sup>Ser</sup>* (AGN) in both mitochondrial genomes lacked the dihydrouridine (DHU) loop. This feature has been shown in the majority of metazoan mitogenomes, including all those sequenced from butterflies (Kim, I. et al. 2006; Hong, G. Y. et al. 2009; Hu et al. 2010; Kim, M. I. et al. 2009; Xia et al. 2011; Wang et al. 2011; Kim, M. J. et al. 2010, 2011a, 2011b; Chen et al. 2012; Shi et al. 2012; Tian et al. 2012). The anticodon for *tRNA<sup>Ser</sup>* (AGN) in mitogenomes of butterfly species was either TCT, GCT, or

ACT, whereas only GCT was detected in all other sequenced mitogenomes of pierid species (*P. rapae*, *A. crataegi*, *D. hyparete*, and *A. melete*) (Hong, G. Y. et al. 2009, Mao et al. 2010, Park et al. 2012, Shi et al. 2012). The tRNA-like structure (*tRNA<sup>Leu</sup>* [UUR]) was detected in the *16S rRNA* gene of *C. pomona*, and a similar observation had been made in *C. vasava* (Hao et al. 2012). Interestingly, the 81 bp insertion of the tRNA-like sequence was made up completely of A and T, without G and C nucleotides. The *L. morsei* and *C. pomona* anticodon sequences of each tRNA isotype were identical to those of all other sequenced butterfly mitogenomes. As in other insects, unmatched base pairs were also detected in the stems of tRNAs. For *L. morsei*, there were 32 unmatched base pairs, consisting of 25 G-U, one A-A, and six U-U mismatches, whereas in *C. pomona*, 21 G-U, two A-A, and six U-U mismatches were identified.

Both of the two pierid mitogenomes harbored a large and a small ribosomal RNA subunit (*16S rRNA* and *12S rRNA*), located between *tRNA<sup>Leu</sup>* (CUN) and *tRNA<sup>Val</sup>*, and between *tRNA<sup>Val</sup>* and the A+T-rich region, respectively. The length of the *16S rRNA* and *12S rRNA* genes in *L. morsei* were 1,337 and 764 bp, respectively, with A+T contents of 84.29 and 83.25%, respectively; the *16S rRNA* and *12S rRNA* in *C. pomona* were 1,332 and 779 bp in length, respectively, with A+T contents of 85.21 and 85.11%, respectively (Table 2).

### Intergenic spacers and overlapping sequences

The mitogenomes of *L. morsei* and *C. pomona* harbored 11 and 15 intergenic spacers, ranging from 1 to 39 bp (94 bp in total) and 1 to 24 bp (87 bp in total), respectively (Table 3). Among these, only three intergenic spacers were longer than 10 bp in both pierid species

(Table 3). The longest intergenic spacers, located between the *tRNA<sup>Gln</sup>* and *ND2* genes in *L. morsei* and *C. pomona*, were 39 and 24 bp in length, respectively, with A+T contents of 90.35 and 91.67% respectively. This spacer is present in all of the butterfly mitogenomes sequenced to date, whereas absent in all non-lepidopteran insects. Another long spacer harboring the 7 bp AACTAA motif, located between the *tRNA<sup>Ser</sup>* (UCN) and *ND1* genes, has been observed commonly in most insect groups including all other butterflies.

In addition, there were 33 overlapping nucleotides scattered over 13 locations in *L. morsei*, and 26 nucleotide overlaps scattered over eight locations in *C. pomona* (Table 2). Among these overlaps in the two pierid species, the longest one was 8 bp in length and located between *tRNA<sup>Trp</sup>* and *tRNA<sup>Cys</sup>* with the 7 bp motif AGCCTTA; the second longest one was 7 bp in length and located between *ATP8* and *ATP6* with the 7 bp motif ATGATAA. Both of these motifs have been observed in the mitogenomes of many butterfly species, including all the other pierids sequenced.

### The A+T-rich region

The A+T-rich regions of *L. morsei* and *C. pomona* were 356 and 313 bp in length, respectively, with A+T contents of 89.60 and 97.13%, respectively. Among the A+T-rich regions of all the butterfly mitogenomes sequenced, that of *C. pomona* was the shortest in length and the highest in A+T content (Table 2). The A+T-rich regions of *L. morsei* and *C. pomona* contained the motif ATAGA, followed by a 19 and 18 bp poly-T stretch, respectively (Fig. 3). Besides, the regions also included microsatellite-like elements, such as (TA)<sub>9</sub>(AT)<sub>3</sub> in *L. morsei* and (TA)<sub>9</sub> in *C. pomona*, which were preceded by the ATTA motif characteristic of lepidopteran mitoge-

nomes. Additionally, a triplicated 23 bp and a duplicated 24 bp repeat element of unknown function were found in *L. morsei* and *C. pomona*, respectively (Fig. 3), and similar repeat elements were detected in other butterflies, such as *A. melete* (Hong, G. Y. et al. 2009), *E. autonoe* (Kim, M. J. et al. 2010), and *Agehana maraho* (Shiraki and Sonan) (Lepidoptera: Papilionidae) (syn. *Papilio maraho*) (Wu et al. 2010).

### Phylogenetic analysis

Several competing hypotheses exist on the phylogenetic family relationships in butterflies. Ehrlich (1967), Ehrlich and Ehrlich (1967), and Scott (1985) demonstrated the close relationship between the Nymphalidae and Lycaenidae and that between the Pieridae and Papilionidae via numerical taxonomic methods and morphological characters. Son and Kim (2011) and Wahlberg et al. (2005) obtained results consistent with the traditional view of the sister relationship between the Pieridae and the Nymphalidae + Lycaenidae group, with Papilionidae as the basal lineage, in agreement with the earlier hypothesis of Kristensen (1976) and supported by several recent studies (Akaike 1974, Kristensen 1976, de Jong et al. 1996, Ackery et al. 1999, Wahlberg et al. 2005, Liao et al. 2010, Kim, M. I. et al. 2010, Kim, M. J. et al. 2011b, Son et al. 2011). However, Chai et al. (2012) and Zhang et al. (2012), on the basis of mitochondrial genomic data, proposed a close relationship between the Pieridae and Lycaenidae, with the Nymphalidae being the sister group, in agreement with the hypothesis of Robbins (1988). Therefore, controversy exists regarding the relationships among the Nymphalidae, Pieridae, and Lycaenidae.

In this study, we conducted phylogenetic analyses via Bayesian inference and maximum likelihood methods, using concatenated

nucleotide datasets of 13 protein-coding genes (6,582 aligned sites, 910 gaps, and 3,746 excluded positions), resulting in similar tree topologies of the butterfly families Papilionidae, Pieridae, Lycaenidae, and Nymphalidae (Fig. 4 A and B). The presented trees showed two major clusters (Fig. 4 A and B). The first one had the Papilionidae as the basal lineage, and the other one included the rest of the butterfly families. Maximum likelihood and Bayesian inference trees suggest a close relationship between Pieridae and Lycaenidae (Fig. 4 A and B), in agreement with the prevailing phylogeny of butterfly families (Hesperiidae (Papilionidae (Nymphalidae (Pieridae, Lycaenidae)))). Although the close relationship of the Pieridae and Lycaenidae proposed herein is contradictory to the traditional view (Pieridae (Nymphalidae, Lycaenidae)), the result is consistent with those of recent studies (Kim, M. J. et al. 2010, Chai et al. 2012, Hao et al. 2012, Zhang et al. 2012). However, uncertainty does exist regarding the sister relationship of the Pieridae and Lycaenidae as shown in the maximum likelihood tree (Fig. 4A). We also note that this relationship has been derived mainly from the protein-coding genes of the mitochondrial genome of butterflies.

In conclusion, although it may still be immature to suggest that the phylogeny of butterfly families is resolved, we do suggest that the sister relationship of the Pieridae and Lycaenidae is supported at the mitogenomic level in this study. We believe that the problem of butterfly phylogeny should be resolved step by step as gene sequence data and morphologic characters are being accumulated, and hopefully more sophisticated analytic tools will become available.

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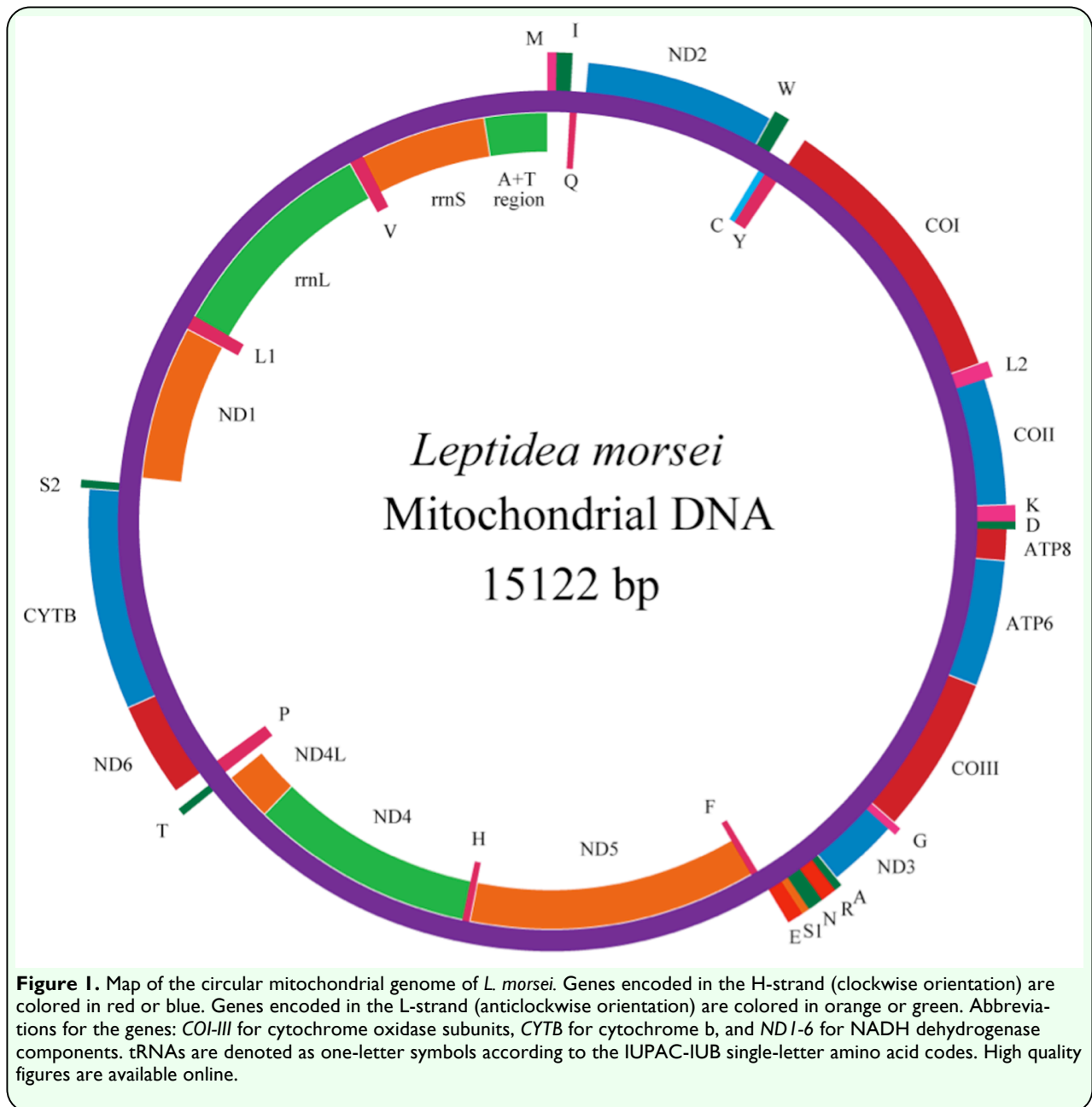
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	←tRNA <sup>Tyr</sup> COI→			
<i>Parnassius bremeri</i>	<u>aa</u> aaaatttacaatttatacgccttatactcagccattttat tag <u>CGA</u>	AAA	TGA	CTA RKWL
<i>Papilio maraho</i>	<u>aa</u> aaaatttacaatttatacgccttatactcagccattttat tag <u>CGA</u>	AAA	TGA	CTT RKWL
<i>Artogeia melete</i>	<u>aa</u> aaaatttacaatttatacgccttataactcagccattttat tag <u>CGA</u>	AAA	TGA	CTT RKWL
<i>Coreana raphaelis</i>	<u>ata</u> aaaatttacaatttatacgccttatactcagccattttat tag <u>CGA</u>	AAA	TGA	CTT RKWL
<i>Spindasis takanonis</i>	<u>aa</u> aaaatttacaatttatacgccttataactcagccattttat tag <u>CGA</u>	AAA	TGA	CTT RKWL
<i>Protantigius superans</i>	<u>ata</u> aaaatttacaatttatacgccttataactcagccattttat tag <u>CGA</u>	AAA	TGA	CTT RKWL
<i>Eumenis autonoe</i>	<u>tt</u> acaatttatacgccttatacctcagccattttactat ttt aag <u>CGA</u>	AAA	TGA	CTT RKWL
<i>Acraea issoria</i>	<u>aa</u> aaaatttacaatttatacgccttataactcagccatttta ttg <u>CGA</u>	AAA	TGA	CTT MRKWL
<i>Sasakia charonda</i>	<u>aa</u> atttacaatttatacgccttataactcagccattttattat aag <u>CGA</u>	AAA	TGA	TTA MTQPFYYKRKWL
<i>Sasakia charonda kuriyamaensis</i>	<u>aa</u> atttacaatttatacgccttataactcagccattttattat aag <u>CGA</u>	AAA	TGA	TTA MTQPFYYKRKWL
<b><i>Leptidea morsei</i></b>	<b><u>aat</u>ttacaatttatacgccttataactcagccattttat ttg <u>CGA</u></b>	AAA	TGA	CTT RKWL
<b><i>Catopsilia pomona</i></b>	<b><u>aat</u>ttacaatttatacgccttataactcagccattttat tag <u>CGA</u></b>	AAA	TGA	CTT RKWL
<i>Ostrinia furnacalis</i>	<u>tt</u> acaatttatacgccttataactcagccattttatttta ttg <u>CGA</u>	AAA	TGA	CTA IWRKWL
<i>Ostrinia nubilalis</i>	<u>tt</u> acaatttatacgccttataactcagccattttatttta ttg <u>CGA</u>	AAA	TGA	CTA IWRKWL
<i>Diatraea saccharalis</i>	<u>ata</u> aaaatttacaatttatacgccttataactcagccattttat tag <u>CGA</u>	AAA	TGA	CTA RKWL
<i>Antheraea pernyi</i>	<u>att</u> tacaatttatacgccttataactcagccattttatttaa ttg <u>CGA</u>	AAA	TGA	ATT RKWI
<i>Antheraea yamamai</i>	<u>tt</u> tacaatttatacgccttataactcagccattttatttaa ttg <u>CGA</u>	AAA	TGA	CTT RKWL
<i>Caligula boisduvalii</i>	<u>att</u> tattaacaagaaaattctttttattaaattat taa ttg <u>CGA</u>	AAA	TGA	CTA MRKWL
<i>Eriogyna pyretorum</i>	<u>t</u> aaaatttacaatttatacgccttataactcagccattttat tag <u>CGA</u>	AAA	TGA	CTA RKWL
<i>Bombyx mandarina</i>	<u>t</u> atcgccttattctcagccattttatttacttactttat ttg <u>CGA</u>	AAA	TGA	ATT RKWI
<i>Bombyx mori</i>	<u>aaa</u> atcgccttattctcagccattttattttttctttt ttg <u>CGA</u>	AAA	TGA	ATT RKWI
<i>Manduca sexta</i>	<u>t</u> aaaatttacaatttatacgccttataactcagccattttatt ttg <u>CGA</u>	AAA	TGA	CTT RKWL
<i>Phthonandria atrilineata</i>	<u>ata</u> aaaatttacaatttatacgccttataactcagccattttat ttg <u>CGA</u>	AAA	TGA	CTA RKWL
<i>Ochrogaster lunifer</i>	<u>tt</u> tacaatttatacgccttataactcagccattttatttttt tag <u>CGA</u>	AAA	TGA	TTA RKWL
<i>Lymantria dispar</i>	<u>tt</u> tacaatttaccgcttataactcagccattttataaaaaat aag <u>CGA</u>	AAA	TGA	TTA RKWL
<i>Fabriciana nerippe</i>	<u>aat</u> ttacaatttatacgccttataactcagccattttatttt ttg <u>CGA</u>	AAA	TGA	CTT MRKWL

**Figure 2.** Alignment of the initiation codons of the *COI* genes of lepidopterans, including those of *L. morsei* and *C. pomona*. The first four or five codons for *COI* and their amino acids are shown on the right-hand side of the figure. Underlined nucleotides indicate the adjacent partial sequence of tRNA<sup>Tyr</sup>. Arrows indicate the transcriptional direction. Boxed nucleotides indicate the currently proposed translation initiators for the *COI* gene of lepidopteran insects. The start codon for *L. morsei* and *C. pomona* is designated as CGA. High quality figures are available online.



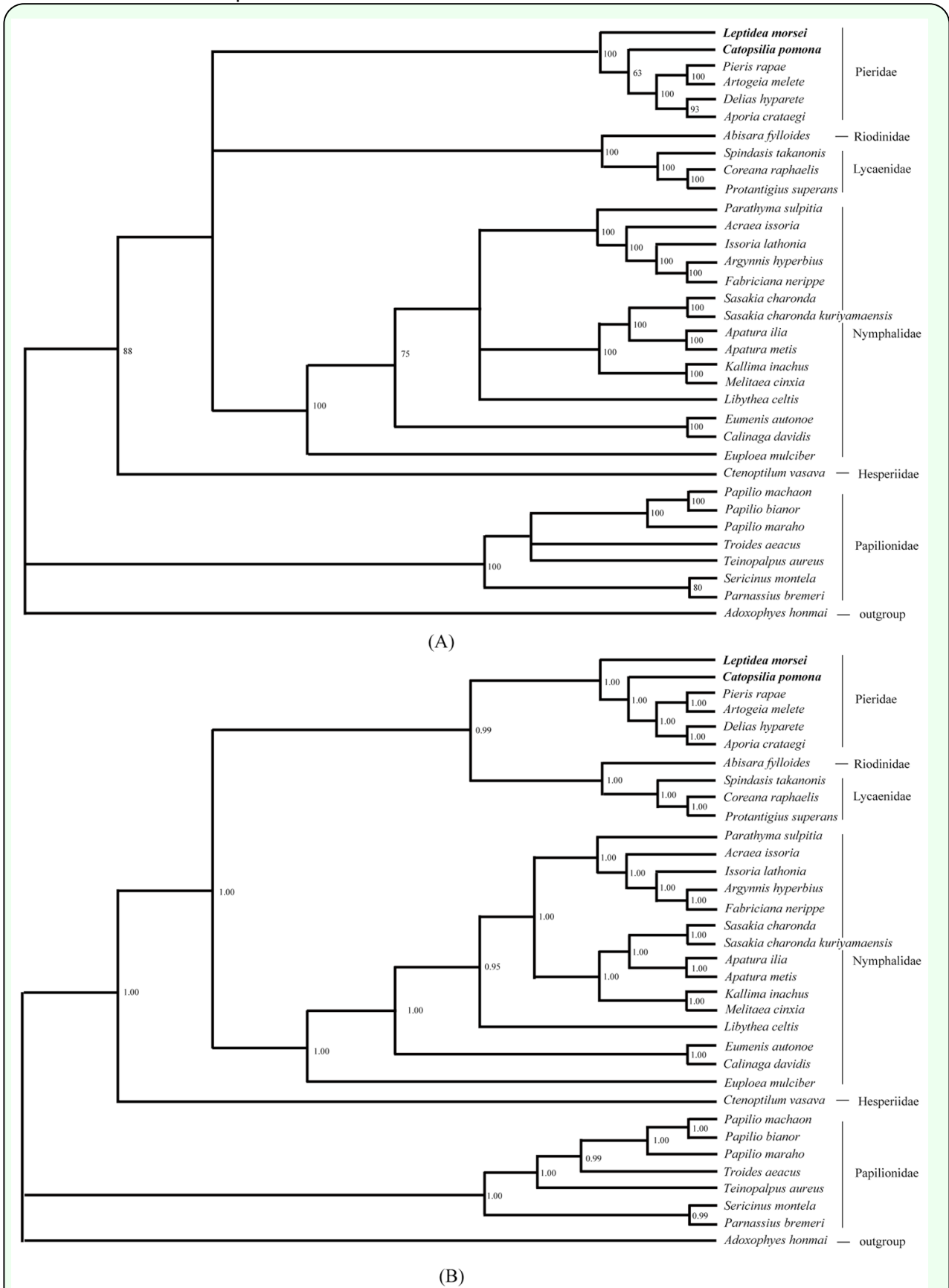
rrnS – 14,829 AATATTTAAAAAATTATAC ATAGATTTTTTTTTTTTTTTT ATATTATATTTAATATAATTATTA AATTTTAAATATT  
 The origin of light strand replication  
 TATCCCTCTTATTTTTTTCCTAATATTCATATTA AAAATTA AATTCATGATAAAGCGATTAATAATCTTTTAAATAGATAACTAT  
 TATTAATAAAAATATATAGGTTTCTAATTAATTATG TATATATATATATATAATATA TAAATATTTA ATATAAATATAAATTATT  
 A micro satellite (TA)<sub>9</sub>(AT)<sub>3</sub>  
ATTTA AATAAATATTATATAAATTTTAAATATTAATAAAATTACATTTAATTTATTTTCATTGCCAATCCCCACAAATTTTCTAATAAT  
 A triplicated 23-bp repeat element  
 AATCAAATAATTAAAT 15,122 – trnM

(A)

rrnS – 14,766 AATATTTAAAAAATTATAC ATAGATTTTTTTTTTTTTTTT ATATTATATTTAATATAATTATTA AATTTTAAATATT  
 The origin of light strand replication  
 TCTATATTTATTTTTTAAATATTTATATAAAAAATAATATAAATATATACAATTAATAATCTAATTAATAAAAAAATTATTATTAA  
TTTATATTAATAAATAAATTTT ATTTATTATTA AATTAATTAATTTATTA TATATATATATATATA ATTATATTAATAAATT  
 A duplicated 24-bp repeat element A micro satellite (TA)<sub>9</sub>  
 AAATATTTAATATAAAAATATATAAACCTTCTAATTTTTTTTTATATA AAAAAAAAAA 15,142 – trnM  
 A poly-A motif

(B)

**Figure 3.** Structures of the A+T-rich regions in the mitogenomes of *L. morsei* (A) and *C. pomona* (B). High quality figures are available online.



**Table 1.** Mitogenomes of the 33 butterfly species used in this study.

Family	Subfamily	Species	GenBank Acc. No.	References
Nymphalidae	Argynniinae	<i>Argyreus hyperbius</i>	JF439070	Wang <i>et al.</i> , 2011
Nymphalidae	Apaturinae	<i>Apatura ilia</i>	JF437925	Chen <i>et al.</i> , 2012
Nymphalidae	Apaturinae	<i>Apatura metis</i>	JF801742	Zhang <i>et al.</i> , 2012
Nymphalidae	Apaturinae	<i>Sasakia charonda kuriyamaensis</i>	AP011825	Hakozaki <i>et al.</i> , unpublished
Nymphalidae	Apaturinae	<i>Sasakia charonda</i>	AP011824	Wang <i>et al.</i> , 2012
Nymphalidae	Calinaginae	<i>Calinaga davidis</i>	HQ658143	Xia <i>et al.</i> , 2011
Nymphalidae	Danainae	<i>Euploea mulciber</i>	HQ378507	Sun <i>et al.</i> , unpublished
Nymphalidae	Heliconiinae	<i>Acraea issoria</i>	GQ376195	Hu <i>et al.</i> , 2010
Nymphalidae	Heliconiinae	<i>Issoria lathonia</i>	HM243590	Qin <i>et al.</i> , unpublished
Nymphalidae	Heliconiinae	<i>Fabriciana nerippe</i>	JF504707	Kim M.J. <i>et al.</i> , 2011a
Nymphalidae	Limenitinae	<i>Parathyma sulphitia</i>	JQ347260	Tian <i>et al.</i> , 2012
Nymphalidae	Libytheinae	<i>Libythea celtis</i>	HQ378508	Sun <i>et al.</i> , unpublished
Nymphalidae	Nymphalinae	<i>Melitaea cinxia</i>	GQ398377	Xu <i>et al.</i> , unpublished
Nymphalidae	Nymphalinae	<i>Kallima inachus</i>	JN857943	Qin <i>et al.</i> , 2012
Nymphalidae	Satyrinae	<i>Eumenis autonoe</i>	GQ868707	Kim M.J. <i>et al.</i> , 2010
Papilionidae	Papilioninae	<i>Teinopalpus aureus</i>	HM563681	Qin <i>et al.</i> , 2012
Papilionidae	Papilioninae	<i>Papilio maraho</i>	GQ868707	Wu <i>et al.</i> , 2010
Papilionidae	Papilioninae	<i>Papilio machaon</i>	HM243594	Xu <i>et al.</i> , unpublished
Papilionidae	Papilioninae	<i>Papilio bianor</i>	NC018040	Xu <i>et al.</i> , unpublished
Papilionidae	Papilioninae	<i>Troides aeacus</i>	EU625344	Jiang <i>et al.</i> , unpublished
Papilionidae	Parnassiinae	<i>Parnassius bremeri</i>	FJ871125	Kim <i>et al.</i> , 2009
Papilionidae	Parnassiinae	<i>Sericinus montela</i>	HQ259122	Ji <i>et al.</i> , 2012
Lycaenidae	Aphnaeinae	<i>Spindasis takanonis</i>	HQ184266	Kim M.J. <i>et al.</i> , 2011b
Lycaenidae	Theclinae	<i>Coreana raphaelis</i>	DQ102703	Kim I. <i>et al.</i> , 2006
Lycaenidae	Theclinae	<i>Protantigius superans</i>	HQ184265	Kim M.J. <i>et al.</i> , 2011b
Riodinidae	Nemeobiinae	<i>Abisara fylloides</i>	HQ259069	Zhao <i>et al.</i> , unpublished
Pieridae	Coliadinae	<i>Catopsilia Pomona</i>	JX274649	This study
Pieridae	Dismorphiinae	<i>Leptidea morsei</i>	JX274648	This study
Pieridae	Pierinae	<i>Artogeia melete</i>	EU597124	Hong G.Y. <i>et al.</i> , 2009
Pieridae	Pierinae	<i>Pieris rapae</i>	HM156697	Mao <i>et al.</i> , 2010
Pieridae	Pierinae	<i>Delias hyparete</i>	JX094279	Shi <i>et al.</i> , 2012
Pieridae	Pierinae	<i>Aporia crataegi</i>	JN796473	Kim <i>et al.</i> , 2012
Hesperiidae	Pyrginae	<i>Ctenoptilum vasava</i>	JF713818	Hao <i>et al.</i> , 2012

**Table 2.** Characteristics of mitogenomes of the 33 butterfly species available.

Taxon	Size (bp)	A+T content (%)	No. codons <sup>a</sup>	PCG <sup>b</sup> A+T (%)	16S rRNA		12S rRNA		A+T-rich region	
					Size (bp)	A+T (%)	Size (bp)	A+T (%)	Size (bp)	A+T (%)
<i>Argyreus hyperbius</i>	15,156	80.8	3,718	79.4	1,330	84.5	778	85.2	349	95.4
<i>Apatura ilia</i>	15,242	80.5	3,711	78.9	1,333	86.0	776	84.9	403	92.5
<i>Apatura metis</i>	15,236	80.5	3,707	78.9	1,333	84.5	779	84.8	394	92.9
<i>Sasakia charonda kuriyamaensis</i>	15,222	79.9	3,586	78.2	1,311	84.2	775	85.0	380	91.8
<i>Sasakia charonda</i>	15,244	79.9	3,695	78.2	1,323	84.4	775	85.0	380	91.8
<i>Calinaga davidis</i>	15,267	80.4	3,737	78.8	1,337	83.8	773	85.9	389	92.0
<i>Euploea mulciber</i>	15,166	81.5	3,713	80.2	1,314	84.6	776	85.3	399	93.5
<i>Acraea issoria</i>	15,245	79.7	3,717	78.1	1,331	83.9	788	83.7	430	96.0
<i>Issoria lathonia</i>	15,172	81.2	3,718	79.9	1,319	84.4	771	85.1	361	96.1
<i>Fabriciana nerippe</i>	15,140	80.9	3,719	79.6	1,321	84.4	773	84.9	329	95.4
<i>Parathyma sulphita</i>	15,282	81.9	3,729	80.6	1,319	84.7	779	85.7	349	94.6
<i>Libythea celtis</i>	15,164	81.2	3,732	80.1	1,335	84.7	774	85.4	328	96.3
<i>Melitaea cinxia</i>	15,170	80.0	3,718	78.6	1,336	84.6	772	84.6	338	92.9
<i>Kallima inachus</i>	15,183	80.3	3,704	79.2	1,335	82.8	774	85.1	376	92.0
<i>Eumenis autonoe</i>	15,489	79.1	3,728	76.8	1,335	83.7	775	85.3	678	94.5
<i>Teinopalpus aureus</i>	15,242	79.8	3,720	78.3	1,320	82.4	781	85.6	395	93.1
<i>Papilio maraho</i>	16,094	80.5	3,717	78.1	1,333	83.7	779	85.5	1,270	93.7
<i>Papilio machaon</i>	15,185	80.3	3,720	79.0	1,319	83.5	772	84.2	362	92.5
<i>Papilio bianor</i>	15,340	80.6	3,719	79.0	1,324	83.4	773	85.4	498	94.0
<i>Troides aeacus</i>	15,263	80.2	3,636	79.1	1,234	83.2	784	84.9	418	89.7
<i>Parnassius bremeri</i>	15,389	81.3	3,734	80.2	1,344	83.8	773	85.1	504	93.6
<i>Sericinus montela</i>	15,242	80.9	3,691	79.8	1,338	83.6	760	84.6	408	94.1
<i>Spindasis takanonis</i>	15,349	82.4	3,719	81.0	1,333	85.6	777	84.7	371	94.6
<i>Coreana raphaelis</i>	15,314	82.7	3,708	81.5	1,330	85.3	777	85.8	375	94.1
<i>Protantigius superans</i>	15,248	81.7	3,712	80.3	1,331	85.0	739	85.5	361	93.6
<i>Abisara fylloides</i>	15,301	81.2	3,740	79.8	1,334	85.4	771	85.6	423	91.0
<b><i>Catopsilia pomona</i></b>	<b>15,142</b>	<b>81.3</b>	<b>3,724</b>	<b>80.0</b>	<b>1,332</b>	<b>85.2</b>	<b>779</b>	<b>85.1</b>	<b>313</b>	<b>97.1</b>
<b><i>Leptidea morsei</i></b>	<b>15,122</b>	<b>80.2</b>	<b>3,713</b>	<b>79.2</b>	<b>1,337</b>	<b>84.3</b>	<b>764</b>	<b>83.2</b>	<b>356</b>	<b>89.3</b>
<i>Artogeia melete</i>	15,140	79.8	3,715	78.4	1,319	83.4	777	86.9	351	88.0
<i>Pieris rapae</i>	15,157	79.7	3,721	78.2	1,330	84.0	764	85.0	393	91.6
<i>Delias hyparete</i>	15,186	79.8	3,703	78.4	1,336	83.7	774	85.1	377	92.0
<i>Aporia crataegi</i>	15,140	81.3	3,708	79.9	1,326	85.4	779	85.5	354	95.2
<i>Ctenoptilum vasava</i>	15,468	80.5	3,698	78.9	1,343	84.1	774	86.4	429	88.1

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

**Table 3.** Summarized characteristics of the mitogenomes of *L. morsei* (*Lm*) and *C. pomona* (*Cp*).

Gene	Direction	Nucleotide No.		Size (bp)		IGNc		Start codon/Stop codon	
		<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>
tRNA <sup>Met</sup>	F	1-64	1-67	64	67	0	3		
tRNA <sup>Ile</sup>	F	65-128	71-134	64	64	-3	1		
tRNA <sup>Gln</sup>	R	126-194	136-204	69	69	39	24		
ND2	F	234-1250	229-1260	1017	1032	-2	6	ATT/TAA	ATT/TAA
tRNA <sup>Trp</sup>	F	1249-1314	1267-1332	66	66	-8	-8		
tRNA <sup>Cys</sup>	R	1307-1368	1325-1386	62	62	4	5		
tRNA <sup>Tyr</sup>	R	1373-1436	1392-1456	64	65	2	2		
COI	F	1439-2969	1459-2989	1531	1531	0	0	CGA/T-tRNA	CGA/T-tRNA
tRNA <sup>Leu</sup> (UUR)	F	2970-3036	2990-3055	67	66	0	0		
COII	F	3037-3712	3056-3731	676	676	0	0	ATG/T-tRNA	ATG/T-tRNA
tRNA <sup>Lys</sup>	F	3713-3783	3732-3801	71	70	-1	-1		
tRNA <sup>Asp</sup>	F	3783-3848	3801-3868	66	68	0	0		
ATP8	F	3849-4007	3869-4030	159	162	-7	-7	ATC/TAA	ATT/TAA
ATP6	F	4001-4684	4024-4701	684	678	-1	-1	ATG/TAA	ATG/TAA
COIII	F	4684-5475	4701-5492	792	792	2	3	ATG/TAA	ATG/TAA
tRNA <sup>Gly</sup>	F	5478-5542	5496-5561	65	66	0	0		
ND3	F	5543-5896	5562-5915	354	354	3	-2	ATA/TAA	ATT/ TAG
tRNA <sup>Ala</sup>	F	5900-5965	5914-5982	66	69	-1	-1		
tRNA <sup>Arg</sup>	F	5965-6027	5982-6048	65	67	11	16		
tRNA <sup>Asn</sup>	F	6039-6103	6065-6130	65	66	-1	1		
tRNA <sup>Ser</sup> (AGN)	F	6103-6163	6132-6191	61	60	-1	1		
tRNA <sup>Glu</sup>	F	6163-6228	6193-6259	66	67	-2	0		
tRNA <sup>Phe</sup>	R	6227-6290	6260-6326	64	67	0	0		
ND5	R	6291-8028	6327-8064	1738	1738	-3	0	ATT/T-tRNA	ATT/ T-tRNA
tRNA <sup>His</sup>	R	8026-8089	8065-8130	64	66	0	0		
ND4	R	8090-9413	8131-9466	1325	1336	12	-4	ATA/T-tRNA	ATA/T-tRNA
ND4L	R	9426-9704	9463-9744	279	282	2	3	ATG/TAA	ATG/TAA
tRNA <sup>Thr</sup>	F	9707-9769	9748-9811	63	64	0	0		
tRNA <sup>Pro</sup>	R	9770-9835	9812-9877	66	66	2	2		
ND6	F	9838-10365	9880-10407	528	528	-1	3	ATT/TAA	ATC/TAA
CytB	F	10365-11513	10411-11565	1149	1155	-2	-2	ATG/TAA	ATG/TAA
tRNA <sup>Ser</sup> (UCN)	F	11512-11578	11564-11629	67	66	16	16		
ND1	R	11595-12533	11646-12584	939	939	1	1	ATG/TAA	ATG/TAA
tRNA <sup>Leu</sup> (CUN)	R	12535-12601	12586-12652	67	67	0	0		
1rRNA	R	12602-13938	12653-13984	1337	1332	0	0		
tRNA <sup>Val</sup>	R	13939-14002	13985-14050	64	66	0	0		
srRNA	R	14003-14766	14051-14829	764	779	0	0		
A+T-rich region	R	14767-15122	14830-15142	356	313	356	313		

IGNc: intergenic nucleotide length, the positive number indicates interval nucleotides (base pairs) between genes, while the negative number indicates the overlapped nucleotides (base pairs) between genes.



**Table 4.** Size proportion of coding genes, intergenic spacers, and the A+T-rich region to the whole genome of the butterflies in this study.

Taxon	Total	Gene		Intergenic spacer		A+T-rich region	
	Size (bp)	Size (bp)	%	Size (bp)	%	Size (bp)	%
<i>Argynnis hyperbius</i>	15,156	14,707	97.0	97	0.6	349	2.3
<i>Apatura ilia</i>	15,242	14,718	96.6	155	1.0	403	2.6
<i>Apatura metis</i>	15,236	14,676	96.3	161	1.1	394	2.6
<i>Sasakia charonda kuriyamaensis</i>	15,222	14,340	93.6	608	4.0	380	2.5
<i>Sasakia charonda</i>	15,244	14,643	96.1	321	2.1	380	2.5
<i>Calinaga davidis</i>	15,267	14,775	96.8	130	0.9	389	2.5
<i>Euploea mulciber</i>	15,166	14,682	96.6	117	0.8	399	2.6
<i>Acraea issoria</i>	15,245	14,746	96.7	88	0.6	430	2.8
<i>Issoria lathonia</i>	15,172	14,733	97.0	107	0.7	361	2.4
<i>Fabriciana nerippe</i>	15,140	14,748	97.2	98	0.6	329	2.2
<i>Parathyma sulphitia</i>	15,268	14,919	96.4	213	1.4	349	2.3
<i>Libythea celtis</i>	15,164	14,766	97.2	97	0.6	328	2.2
<i>Melitaea cinxia</i>	15,170	14,751	97.1	96	0.6	338	2.2
<i>Kallima inachus</i>	15,183	14,756	97.0	88	0.6	376	2.5
<i>Euploea mulciber</i>	15,166	14,682	96.6	117	0.8	399	2.6
<i>Teinopalpus aureus</i>	15,242	14,713	96.5	107	0.7	395	2.6
<i>Papilio maraho</i>	16,094	14,705	91.4	111	0.7	1270	7.9
<i>Papilio machaon</i>	15,185	14,733	97.0	125	0.8	362	2.4
<i>Papilio bianor</i>	15,340	14,742	96.1	127	0.8	498	3.2
<i>Troides aeacus</i>	15,263	14,433	94.4	446	2.9	418	2.7
<i>Parnassius bremeri</i>	15,389	14,745	95.8	138	0.9	504	3.3
<i>Sericinus montela</i>	15,241	14,667	96.2	166	1.1	408	2.7
<i>Spindasis takanonis</i>	15,349	14,743	96.1	219	1.4	371	2.4
<i>Coreana raphaelis</i>	15,314	14,689	95.9	177	1.2	375	2.4
<i>Protantigius superans</i>	15,248	14,663	96.2	217	1.4	361	2.4
<i>Abisara fylloides</i>	15,301	14,820	96.5	118	0.8	423	2.8
<b><i>Catopsilia pomona</i></b>	<b>15,142</b>	<b>14,770</b>	<b>97.4</b>	<b>87</b>	<b>0.6</b>	<b>313</b>	<b>2.1</b>
<b><i>Leptidea morsei</i></b>	<b>15,122</b>	<b>14,707</b>	<b>97.0</b>	<b>94</b>	<b>0.6</b>	<b>356</b>	<b>2.3</b>
<i>Artogeia melete</i>	15,140	14,677	96.9	117	0.8	351	2.3
<i>Pieris rapae</i>	15,157	14,682	96.9	112	0.7	393	2.6
<i>Delias hyparete</i>	15,186	14,699	96.8	153	1.0	377	2.5
<i>Aporia crataegi</i>	15,140	14,677	96.9	111	0.7	354	2.3
<i>Ctenoptilum vasava</i>	15,468	14,887	96.1	179	1.2	429	2.8

**Table 5.** Nucleotide compositions in *L. morsei* (*Lm*) and *C. pomona* (*Cp*).

Feature	A (%)		C (%)		G (%)		T (%)		A+T (%)	
	<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>
Whole genome	38.51	39.45	11.83	11.07	7.93	7.64	41.73	41.84	80.24	81.29
Protein-coding genes <sup>a</sup>	34.09	34.35	10.39	9.56	10.46	10.36	45.07	45.67	79.16	80.02
1st codon positions	37.95	37.65	10.48	9.56	15.35	15.84	36.22	36.95	74.17	74.60
2st codon positions	22.25	22.13	15.84	16.03	13.14	13.13	48.77	48.71	71.02	70.84
3st codon positions	42.07	43.29	4.85	3.22	2.88	2.09	50.20	51.40	92.27	94.69
tRNA genes	39.69	41.24	10.93	7.83	8.36	10.86	41.02	40.07	80.71	81.31
rrnL genes	45.62	43.84	5.46	5.18	10.25	9.61	38.67	41.37	84.29	85.21
rrnS genes	44.50	46.34	5.63	4.88	11.13	10.01	38.74	38.77	83.24	85.11
A+T-rich region	41.29	46.65	7.58	2.56	2.81	3.19	48.31	50.48	89.60	97.13

<sup>a</sup> Stop codons excluded.

**Table 6.** The 13 protein-coding gene initiation and termination codons in the mitogenomes of the 33 butterfly species in this study.

Species	Predicted initiation and termination codons												
	ND2	COI	COII	ATP8	ATP6	COIII	ND3	ND5	ND4	ND4L	ND6	CytB	ND1
<i>Argyreus hyperbius</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATA/TAA	ATG/TAA	ATG/TAA	ATA/TAA	ATG/TAA	ATA/TAA
<i>Apatura ilia</i>	ATT/TAA	CGA/TAA	ATG/T	ATT/TAA	ATG/TAG	ATG/TAA	ATA/T	ATT/T	ATG/T	ATG/TAG	ATA/TAA	ATG/TAA	ATG/TAA
<i>Apatura metis</i>	ATT/TAA	CGA/TAA	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATT/T	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
<i>Sasakia charonda kuriyamaensis</i>	ATA/TAA	ATA/TAA	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATA/TAG	ATT/TAA	ATA/TAA	ATA/TAA	ATA/TAA	ATG/TAG	ATG/TAA
<i>Sasakia charonda</i>	ATA/TAA	TTG/TAA	GTG/T	ATC/TAA	ATG/TA	ATG/TAA	ATT/T	ATT/TAA	ATG/TAA	ATG/TA	ATA/TAA	ATG/TAA	ATG/TAA
<i>Calinaga davidis</i>	ATT/TAA	CGA/T	ATG/TAA	ATC/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/TAA	ATG/TA	ATG/TAA	ATT/TAA	ATG/TAA	ATG/TAA
<i>Euploea mulciber</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATT/T	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
<i>Acraea issoria</i>	ATT/TAA	TTG/T	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATA/TAA	ATG/TAA	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
<i>Issoria lathonia</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATG/T	ATT/T	ATT/TAA	ATG/TA	ATT/TAA	ATG/TAA	ATG/TA
<i>Fabriciana nerippe</i>	ATT/T	TTG/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/TA	ATG/TA	ATG/TAA	ATT/TAA	ATG/TAA	ATA/TAA
<i>Parathyma sulphita</i>	ATT/TAA	CGA/TAA	ATG/TAA	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/TAA	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
<i>Libythea celtis</i>	ATC/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/T	ATG/T	ATG/TAG	ATT/TAA	ATG/TAA	ATG/TAA
<i>Melitaea cinxia</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/TAA	ATG/TA	ATG/TAA	ATT/TAA	ATG/TAA	ATG/TA
<i>Kallima inachus</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATA/TAG	ATG/TAA	ATG/TA	ATTG/TAA	ATT/TAA	ATG/TAA	ATG/TAG
<i>Eumenis autonoe</i>	ATT/TAA	CGA/TAA	ATG/T	ATC/TAA	ATG/TAA	ATG/TAA	ATC/TAA	ATC/T	ATG/T	ATG/TAA	ATT/TAA	ATG/TAA	ATG/TAA
<i>Teinopalpus aureus</i>	ATT/T	TAGCGA/T	ATG/T	ATT/TAA	ATG/TAA	ATA/T	ATT/T	ATA/TAA	ATG/T	ATG/TAG	ATT/TAA	ATA/T	ATG/TAG
<i>Papilio maraho</i>	ATT/T	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATA/TAA	ATT/TAA	ATG/T	ATG/TAA	ATC/TAA	ATA/T	ATG/TAG
<i>Papilio machaon</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATT/TAA	ATG/TAA	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAG
<i>Papilio bianor</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATC/TAG	ATT/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAG
<i>Troides aeacus</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TGG	ATA/TAG	ATT/TAA	ATC/TAT	ATG/TAA	ATT/TAA	ATA/TAA	ATG/TAA
<i>Parnassius bremeri</i>	ATT/TAA	CGA/TAA	ATG/T	ATA/TAA	ATG/TAA	ATG/TAA	ATA/TAA	ATT/TAA	ATG/TAA	ATG/TAA	ATC/TAA	ATA/TAA	ATG/TAA
<i>Sericinus montela</i>	ATT/TAA	CGA/T	ATG/TAA	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATT/TAA	ATG/TAA	ATA/TAG	ATT/TAA	ATA/TAA	ATA/TAA
<i>Spindasis takanonis</i>	ATT/TAA	CGA/TAA	ATG/T	ATC/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/TAA	ATG/TAG	ATG/TAA	ATT/TAA	ATG/TAA	ATG/TA
<i>Coreana raphaelis</i>	ATT/TAA	TTAG/T	ATG/T	ATC/TAA	ATG/TA	ATG/TAA	ATT/TAA	ATT/T	ATG/T	ATG/TA	ATA/TA	ATG/T	ATG/TAA
<i>Protantigius superans</i>	ATT/TAA	CGA/TAA	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/T	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
<i>Abisara fylloides</i>	ATT/TAA	CGA/T	ATG/TAA	ATC/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/T	ATG/T	ATG/T	ATA/TAA	ATG/TAA	ATG/TAA
<i>Catopsilia pomona</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATT/T	ATA/T	ATG/TAA	ATC/TAA	ATG/TAA	ATG/TAA
<i>Leptidea morsei</i>	ATT/TAA	CGA/T	ATG/T	ATC/TAA	ATG/TAA	ATG/TAA	ATA/TAA	ATT/T	ATA/T	ATG/TAA	ATT/TAA	ATG/TAA	ATG/TAA
<i>Artogeia melete</i>	ATT/T	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/T	ATG/TAA	ATA/TAA	ATT/TAA	ATG/TAA	ATA/TAA
<i>Pieris rapae</i>	ATT/TAA	TTAAAG/T	ATG/TAA	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATT/TAG	ATG/TAA	ATG/TAA	ATT/TAA	ATG/TAA	ATA/TAA
<i>Delias hyparete</i>	ATT/TAA	CGA/T	ATG/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATT/T	ATG/T	ATA/TAA	ATT/TAA	ATG/TAA	ATA/TAA
<i>Aporia crataegi</i>	ATT/TAA	ATT/TAA	ATG/T	ATC/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATT/T	ATG/TA	ATG/T	ATC/TAA	ATG/TAA	ATG/TAA
<i>Ctenoptilum vasava</i>	ATT/TAG	ATT/TAA	ATG/T	ATA/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATA/TAA	ATG/TAA	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA

**Table 7.** The codon usage in the mitogenomes of *L. morsei* (*Lm*) and *C. pomona* (*Cp*).

Amino acid	Codon	N		RSCU		Amino acid	Codon	N (%)		RSCU	
		<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>			<i>Lm</i>	<i>Cp</i>	<i>Lm</i>	<i>Cp</i>
Phe	UUU	338	349	1.82	1.87	Tyr	UAU	169	177	1.81	1.84
	UUC	34	25	0.18	0.13		UAC	18	15	0.19	0.16
Leu	UUA	454	470	4.96	5.31	His	CAU	59	59	1.79	1.79
	UUG	11	9	0.12	0.10		CAC	7	7	0.21	0.21
	CUU	52	33	0.57	0.37	Gln	CAA	61	63	1.94	2.00
	CUC	4	3	0.04	0.03		CAG	2	0	0.06	0.00
	CUA	28	16	0.31	0.18	Asn	AAU	247	241	1.84	1.91
	CUG	0	0	0.00	0.00		AAC	21	12	0.16	0.09
Ile	AUU	435	444	1.86	1.93	Lys	AAA	93	103	1.82	1.87
	AUC	33	15	0.14	0.07		AAG	9	7	0.18	0.13
Met	AUA	283	298	1.88	1.86	Asp	GAU	52	57	1.73	1.78
	AUG	18	22	0.12	0.14		GAC	8	7	0.27	0.22
Val	GUU	68	66	2.25	2.03	Glu	GAA	68	71	1.70	1.87
	GUC	1	3	0.03	0.09		GAG	12	5	0.30	0.13
	GUA	47	58	1.55	1.78	Cys	UGU	21	28	1.62	1.81
	GUG	5	3	0.17	0.09		UGC	5	3	0.38	0.19
Ser	UCU	102	110	2.56	2.83	Trp	UGA	89	97	1.85	2.00
	UCC	10	5	0.25	0.13		UGG	7	0	0.15	0.00
	UCA	85	86	2.13	2.21	Arg	CGU	15	19	1.09	1.46
	UCG	2	2	0.05	0.05		CGC	0	1	0.00	0.08
CCU	72	74	2.38	2.41	CGA		38	31	2.76	2.38	
Pro	CCC	9	9	0.30	0.29	CGG	2	1	0.15	0.08	
	CCA	39	40	1.29	1.30	Ser	AGU	41	32	1.03	0.82
	CCG	1	0	0.03	0.00		AGC	3	1	0.08	0.03
	ACU	76	95	2.03	2.50		AGA	76	72	1.91	1.85
Thr	ACC	9	7	0.24	0.18	AGG	0	3	0.00	0.08	
	ACA	65	50	1.73	1.32	Gly	GGU	47	55	0.98	1.09
	ACG	0	0	0.00	0.00		GGC	5	1	0.10	0.02
	GCU	70	75	2.37	2.52		GGA	102	120	2.14	2.39
Ala	GCC	13	6	0.44	0.20	GGG	37	25	0.77	0.50	
	GCA	34	37	1.15	1.24	Stop	UAA	0	0	0.00	0.00
	GCG	1	1	0.03	0.03		UAG	0	0	0.00	0.00

Start and stop codons excluded from total codon counts; N, frequency of codon use; RSCU, relative synonymous codon usage.