

ORIGINAL ARTICLE

The complete mitochondrial genome of *Oreta fuscopurpurea* (Lepidoptera: Drepanidae) and its phylogenetic implications

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Abstract In this study, the first complete mitochondrial genome (mitogenome) of lepidopteran family Drepanidae (superfamily Drepanoidea) was reported with the notes about its phylogenetic implications. The *Oreta fuscopurpurea* mitogenome is 15,564 bp in length, including 13 protein-coding genes, two ribosomal RNAs (1rRNA and srRNA), 22 transfer RNAs and a non-coding control region (AT-rich region), with a 79.6% A+T content. The gene orientation and arrangement of the mitogenome are the same as other sequenced lepidopterans. All protein-coding genes usually start with the common ATN codon except for COI gene, which used CGA as the initial codon; eight PCGs use a typical stop codon of TAA, whereas the remaining PCGs use incomplete stop codon of T or TA. All tRNAs have the typical clover-leaf structure with the exception of tRNA^{Ser} (AGN). The 1rRNA and srRNA genes are 1,409 bp and 778 bp in size respectively, with the former harboring one (TA)₁₃ microsatellite-like repeat and an 17 bp insertion. The 20 intergenic spacers totaling of 184 bp and 8 overlapping sequences totaling of 25 bp are scattered throughout the whole mitogenome. The 526 bp AT-rich region contains some structures characteristic of lepidopterans, such as the motif ATAGA preceded by an 19 bp poly-T stretch, a tRNA-like and a stem-loop structures. Phylogenetic analysis of the *Oreta fuscopurpurea* with other 47 insect species covering 20 lepidopteran families were conducted based on the sequence data of the 13 mitogenomic protein coding genes with maximum likelihood (ML) and Bayesian inference (BI) methods, and the results showed distinctly that the superfamily Drepanoidea was sister to the clade of (Bombycoidea, Lasiocampoidea) + (Noctuoidea, Geometroidea).

Key words Drepanoidea, mt DNA, tRNA-like sequence, phylogenetic analysis.

1 Introduction

Insect mitochondrial genomes (mitogenomes) are usually circular molecules ranging from 14 to 19 kb in size. They usually contain 13 protein-coding genes, two ribosomal RNA genes (1rRNA and srRNA), 22 tRNAs and a AT-rich region (Wolstenholme, 1992; Zhang & Hewitt, 1997; Boore, 1999). Compared with nuclear genes, mitogenomes have its own unique features such as the maternal inheritance, high evolutionary rate and lack of recombination (Cameron & Whiting, 2008), and thus have become one of the most powerful tools in the study areas of systematics and phylogenetics, such as genetic resources, population genetics, molecular evolution, phylogeography, etc. (Boore, 1999; Avise *et al.*, 2000; Zakharov *et al.*, 2004; Hurst & Jiggins, 2005; Kim *et al.*, 2005; Cameron & Whiting, 2008; Liu *et al.*, 2013; Timmermans *et al.*, 2014).

The Lepidoptera is the second largest insect order exceeding 30 superfamilies covering more than 160,000 species (Kristensen *et al.*, 2007; van Nieukerken *et al.*, 2011). So far, the phylogenetic relationships among the main lepidopteran

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lineages, especially about the superfamily Drepanoidea with other lepidopterans has not been fully resolved from comprehensively morphological and molecular views. For example, Minet (1983) proposed that the Drepanoidea was the sister group of Geometroidea based on some pupal characters and some other morphological evidences (Kristensen *et al.*, 2003); Regier *et al.* (2009) suggested that Drepanoidea is not a monophyletic group; Mutanen (2010) proposed that the core of Drepanidae associating with Doidae and Cimelioidae (for Epicopeiidae falling outside) form a sister group to Lasiocampoidea; Bazinet *et al.* (2013) concluded that the Drepanoidea (including families of Doidae, Cimeliidae and Drepanidae) was the sister group of the remaining four Macroheterocera superfamilies (Geometroidea, Bombycoidea, Lasiocampoidea, Noctuoidea) based on transcriptome RNA-Seq data; Heikkilä (2015) revealed that they were sister to the clade of (Bombycoidea + Lasiocampoidea) + Noctuoidea. Thus, Macroheterocera in this modified sense consists of Macrolepidoptera *sensu* minus the expanded concept of the butterflies Papilionoidea *sensu* (Kristensen *et al.*, 2003), including the Bombycoidea, Geometroidea, Noctuoidea, Lasiocampoidea and Drepanoidea; the Papilionoidea, Pyraloidea, together with the Macroheterocera are grouped as the Obtectomera which constitute the Apoditrysia with the Tortricoidea (van Nieukerken *et al.*, 2011).

Up to date, more than 800 complete or nearly complete mitogenomes of 226 lepidopteran species covering 13 superfamilies have been released in GenBank. However, the majority of these mitogenomes are from the superfamilies Noctuoidea, Bombycoidea and Papilionoidea (Regier *et al.*, 2009; Mutanen *et al.*, 2010; Zwick *et al.*, 2011; Tian *et al.*, 2012), only with a few from other superfamilies or families. In this study, we firstly determined the complete mitogenome sequence of *Oreta fuscopurpurea*, a member of the family Drepanidae (superfamily Drepanoidea), and compared its features with those of other reported representative lepidopteran species. In addition, we reconstructed the phylogenetic trees of 44 lepidopteran species covering nine lepidopteran superfamilies (van Nieukerken *et al.*, 2011) based on the 13 PCG nucleotide sequence data using maximum likelihood and Bayesian inference methods, in order to further investigate the phylogenetic relationships of the superfamily Drepanoidea with other major lineages of Macroheterocera.

2 Materials and methods

2.1 Sample collection and DNA extraction

The adult individuals of *Oreta fuscopurpurea* (Lepidoptera: Drepanoidea: Drepanidae) were collected in Xialing Scenic Area, Xuancheng, Anhui Province, China, in August 2016. After species identification, the materials were conserved in 100% ethanol rapidly for DNA fixation and kept at -20°C for DNA extraction subsequently. Total genomic DNA was extracted from thorax musculature using a DNA extraction kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instruction.

2.2 PCR amplification and sequencing

The complete mitochondrial genome of *O. fuscopurpurea* was amplified by PCR amplification using universal primers (Simon *et al.*, 1994, 2006; Zhao *et al.*, 2011) and some specific primers designed by Primer Premier 5.0 software (Singh *et al.*, 1998). These primers were all synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The long fragments were amplified using LA Taq DNA polymerase (Takara Bio, Otsu, Shiga, Japan) under the following conditions: 5 min at 95°C ; followed by 30 cycles of 55s at 95°C , 2 min at 42°C – 45°C and 2.5 min at 68°C ; final elongation for 10 min at 68°C ; short fragments were then amplified with conditions: 5 min at 95°C ; followed by 35 cycles of 50s at 94°C , 50s at 41°C – 52°C and 1.5 min at 72°C ; final elongation for 10 min at 72°C . The PCR products were separated by a 1.2% agarose gel electrophoresis, purified using DNA gel extraction kit (TaKaRa), and then sequenced by Sangon Biotech Co., Ltd (Shanghai, China).

2.3 DNA sequence annotation and analysis

The sequences of PCGs and rRNAs were identified by using the NCBI BLAST program, then by alignment with other lepidopteran sequences by applying MEGA 7.0 (Kumar *et al.*, 2016) and Clustal X 1.83 (Thompson *et al.*, 1997) softwares. The sequences of PCGs were translated via invertebrate mitogenome genetic code. The tRNAs and potential second structures were identified by using the tRNAscan-SE 2.0 (Lowe & Chan, 2016). And the remaining tRNAs that could not be found by the tRNAscan-SE 2.0 were confirmed by sequence comparisons with other reported lepidopterans. All tRNAs and rRNAs were folded and drawn manually. Composition skewness of PCGs and rRNAs were measured by the formulas: AT

skew=(A-T)/(A+T); GC skew=(G-C)/(G+C) (Perna & Kocher, 1995). The tandem repeats of the A+T region were predicted by the Tandem Repeats Finder (Benson, 1999).

2.4 Phylogenetic analysis

Phylogenetic relationships of *O. fuscopurpurea* with other 43 lepidopteran species were reconstructed based on mitogenomic PCG sequence data with maximum likelihood (ML) and Bayesian inference (BI) methods using one Trichoptera species *Hydropsyche pellucidula* (NC_029246.1), two Diptera species *Ceratitis fasciventris* (NC_035497.1) and *Aedes albopictus* (KX_809764.1) as the outgroups. The nucleotide sequences of 13PCGs were aligned with CLUSTAL X using default settings. The ML and Bayesian inference analyses were conducted by the softwares PAUP* version 4.0b10 (Swofford, 2002) and MrBayes 3.2.5 (Ronquist *et al.*, 2012), respectively. Evolutionary model was calculated using the program of Modeltest ver. 3.7 (Posada & Crandall, 1998) by the Akaike information criterion, and the resultant optimal model (GTR+I+G) was selected. In maximum likelihood analysis, the trees were searched through the TBR branch swapping method and to obtain the support values of each tree node by 1,000 bootstrap replicates. In Bayesian analysis, two simultaneous runs were conducted for 1,000,000 generations with sampling each 100 generations (each run contains three hot and one cold chains). When the convergence of MCMC chains were achieved (the average standard deviation of the split frequencies was less than 0.01), the first 25% burn-in samples were discarded. The confidence values of the BI tree were expressed as the Bayesian posterior probabilities.

3 Results and discussion

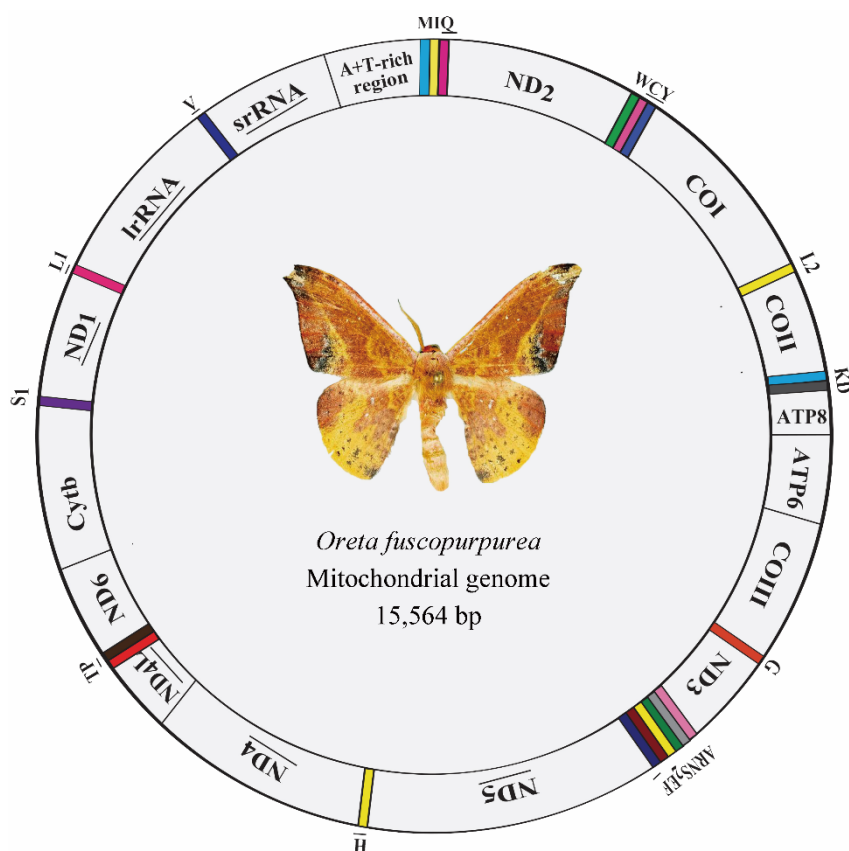


Figure 1. Map of the mitogenome of *O. fuscopurpurea*. The tRNA genes are labeled according to the IUPAC-IUB. The abbreviations for the genes are as follows: COI, COII, and COIII refer to the cytochrome oxidase subunits, Cytb refers to cytochrome B, ATP6 and ATP8 refer to subunits 6 and 8 of ATPase, and ND1-6 refers to components of NADH dehydrogenase. The tRNAs are denoted by color blocks.

3.1 Genome organization

The complete mitogenome of *Oreta fuscopurpurea* is 15,564 bp in length, and contains typical 37 genes for insects: 13 PCGs, two ribosomal RNAs (1rRNA and srRNA), 22 tRNAs, and an AT-rich region (Fig. 1). The gene order and orientation are identical to other sequenced lepidopterans except for some *Thitarodes* species (Boore *et al.*, 1998; Cao *et al.*, 2012). The A+T content is 79.6% for the whole mitogenome, whereas are 77.8%, 82.3%, 86.3%, and 92.9% for the 13 PCGs, 1rRNA, srRNA and the A+T-rich region, respectively. These values are consistent with the corresponding values found in other lepidopteran mitogenomes (Table 1). The complete mitogenome of *O. fuscopurpurea* has been deposited into GenBank under the accession number MG572766.

3.2 Protein-coding genes

All protein-coding genes start with ATN codons (ND2, ND3 with ATT; COII, ATP6, COIII, ND4, ND4L, Cytb with ATG; ND5, ND6, ND1 with ATA), with the exception of cytochrome oxidase subunit I (COI), which used CGA as initiation codon (Table 2). This phenomenon is also found in other lepidopterans, such as the moth *Phalera flavescentis* and *Bombyx mori* (Liu *et al.*, 2013; Sun *et al.*, 2012) and butterfly *Parnassius epaphus* (Wang *et al.*, 2015). Eight of 13 protein-coding genes use the stop codon (TAA), with the remaining PCGs using T or TA as their stop codons. The similar cases are also found in other lepidopterans (Kim *et al.*, 2009; Wang *et al.*, 2015). The base composition analysis of the 13 PCGs showed that the AT content of the first, second and third codon positions are 73.5%, 70.9% and 89.3%, respectively. The relative synonymous codon usages of NNU and NNA codons are fundamentally greater than the others (Table 3), for example, UUU (Phe), UUA (Leu), AUU (Ile), AUA (Met), and AAU (Asn) are the most frequently used codons, accounting for 45.14% of all codons in the mitogenome.

3.3 Transfer and ribosomal RNAs

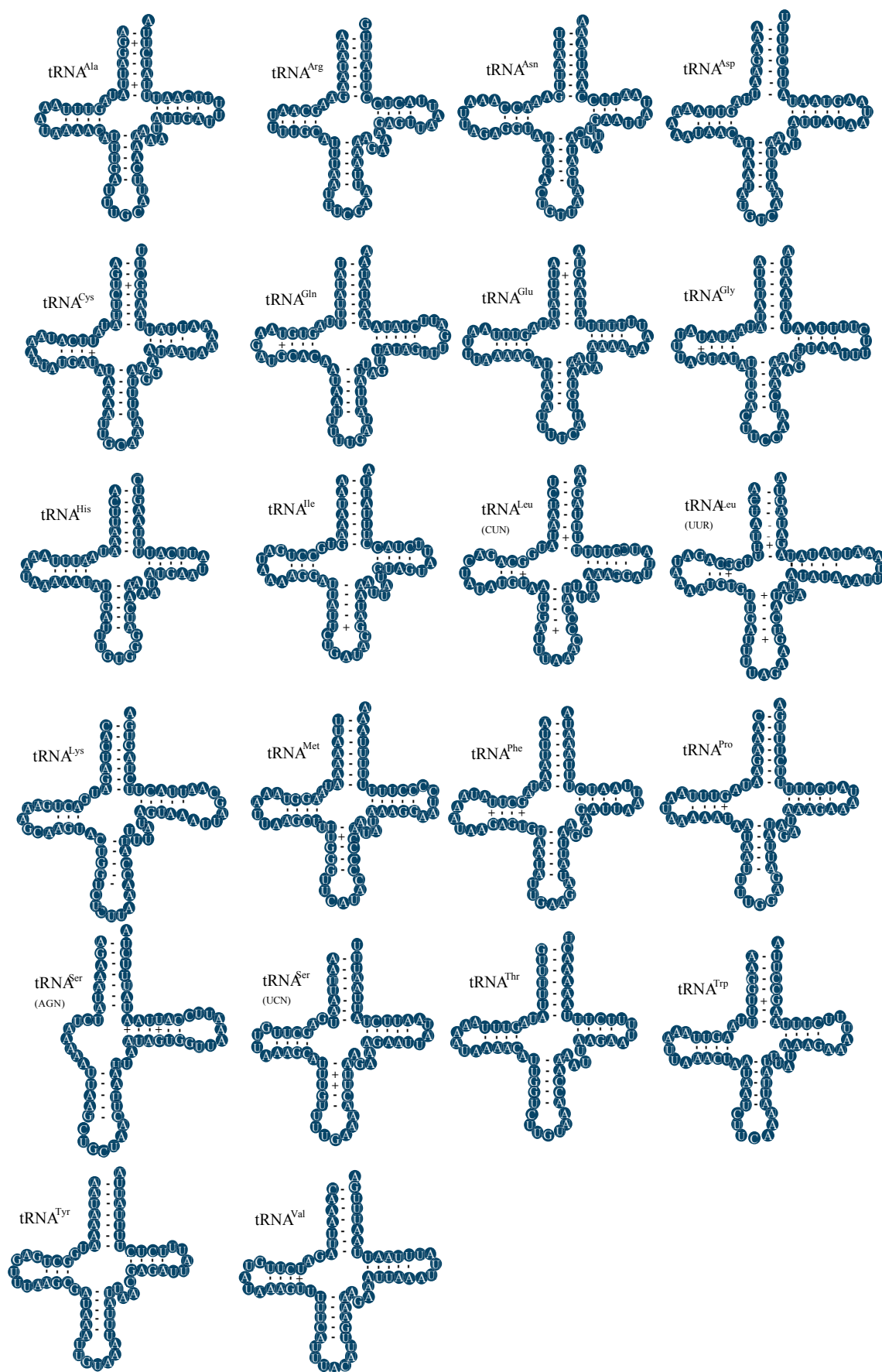
Total 22 typical transfer RNA genes are interspersed the whole genome ranging from 63 bp to 71bp (Fig. 2). They can be folded into the typical cloverleaf secondary structures, except for tRNA^{Ser} (AGN) lacking the DHU loop (Fig. 2). Among these tRNAs, eight are encoded by the light strand and the remaining fourteen by the heavy strand. A total of 25 mismatched base pairs (fifteen U-G, seven U-U, one A-A, one A-C and one U-C) are detected in fifteen tRNAs (seven at the amino acid acceptor stems, nine at the DHU stems, seven at the anticodon stems, and two at the pseudouridine stems). Unmatched base pairs are commonly existed in insect mitochondrial tRNA genes which can be modified via RNA-editing mechanisms (Lavrov *et al.*, 2000).

The 778 bp srRNA and 1,409 bp 1rRNA are located between tRNA^{Val} and the A+T-rich region, and between tRNA^{Leu} (CUN) and tRNA^{Val}, respectively. The former contains three domains with thirty-three helices (Fig. 4), while the later possesses six domains with forty-nine helices (Fig. 3). To our great interests, one (TA)₁₃ microsatellite-like repeat is presented in the 1rRNA which is located between Domains II and IV, whereas the (TA)_n in other lepidopterans is located in the loop region of H2347 (Cameron & Whiting, 2008; Gong *et al.*, 2012; Cameron, 2014; He *et al.*, 2015); in addition, an 17 bp insert region is detected in the 1rRNA located between the stem of H2077/G3 and H2064/G2, while this insert region is found to be inside the highly variable region of H2347/G13 in other lepidopteran species (Cameron & Whiting, 2008; Niehuis *et al.*, 2006). The srRNA secondary structure is generally similar to other sequenced lepidopterans (Wang *et al.*, 2015; He *et al.*, 2015).

3.4 Intergenic spacers and overlapping regions

Twenty intergenic spacers (184 bp) varying from 1 to 58 bp are dispersed throughout the whole genome. Among them, the largest is 58 bp in size and located between tRNA^{Gln} and ND2, with a relatively high A+T content (87.9%). the second largest is 23 bp in size located between tRNA^{Val} and srRNA; the third longest is 20 bp long located between ND3 and tRNA^{Ala}; the fourth and fifth longest spacers are 13 bp and 11 bp, located between tRNA^{Ser} and ND1, between tRNA^{Ser1} and tRNA^{Glu}, respectively. The remaining spacers are all less than 10 bp.

Eight overlapping sequences (totaling of 25 bp) ranging from 1 to 8 bp are detected in the mitogenome. The 8 bp longest (AAGCCTTA) is located between tRNA^{Trp} and tRNA^{Cys}, which case is the same with *P. epaphus* (Wang *et al.*, 2015). The 7 bp second longest (ATGATAA) is located between the ATP6 and ATP8, which is common to other lepidopterans, as well as some non-lepidopteran animals (Boore, 1999).

Figure 2. Predicted cloverleaf secondary structure of the *O. fuscopurpurea* 22 tRNA genes.

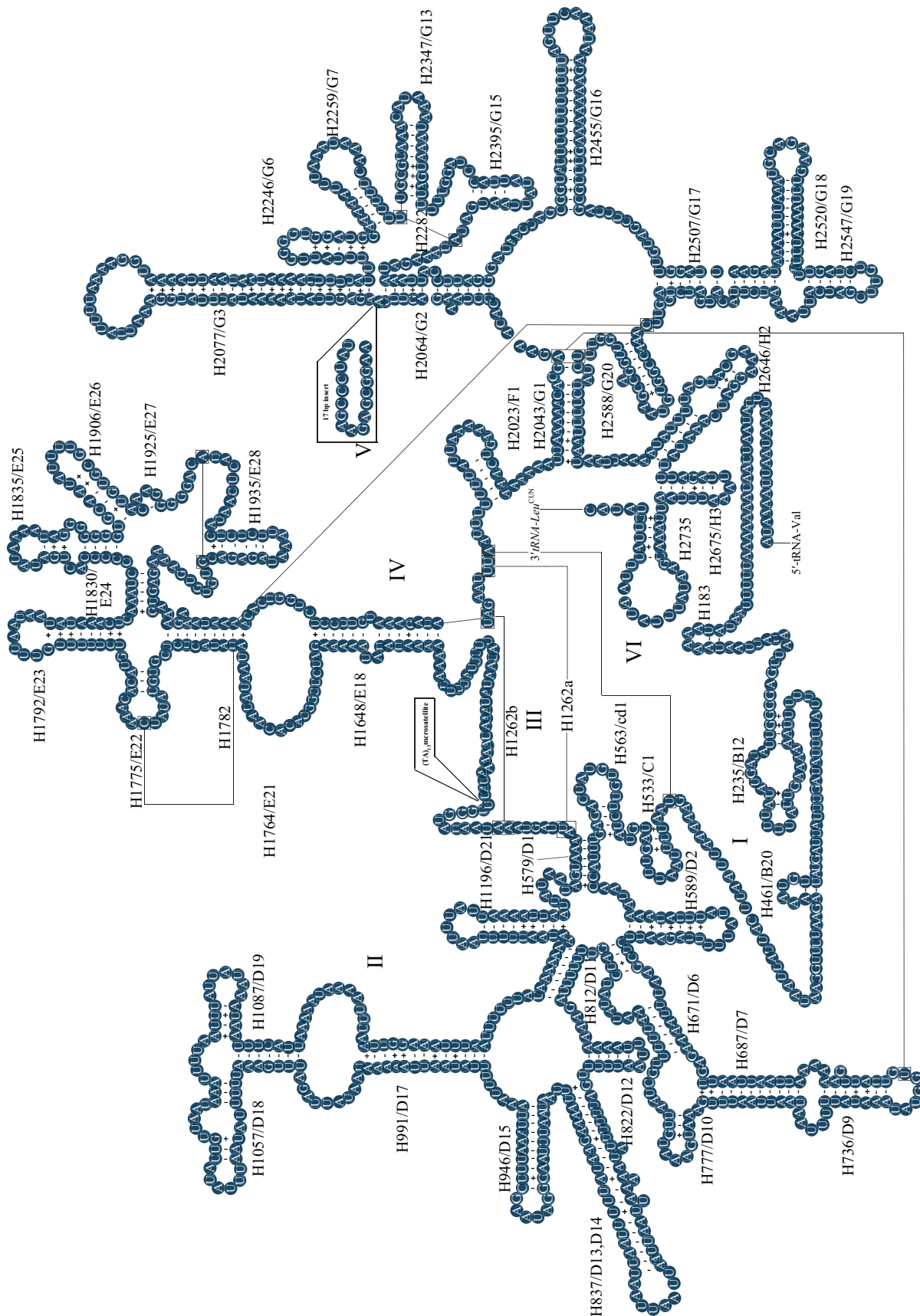


Figure 3. Predicted secondary structure of the *O. fuscopurpurea* lrRNA. Roman numerals denote the conserved domain structure. Helices are numbered according to the annotation systems of Gillespie *et al.* (2006) and Cameron & Whiting (2008). Tertiary structures are denoted by boxed bases joined by solid lines. Watson-Crick pairs are joined by minus signs (-), other interaction are joined by plus signs (+).

Table 1. Characteristics of the lepidopteran mitogenomes in this study.

Taxon	Mitogenome (majority strand)				PCG ^b	rRNA		AT-rich region		GenBank accession No.	
	Size (bp)	AT (%)	AT-skew	GC-skew		NO codons ^a	AT (%)	Size (bp)	AT (%)		Size (bp)
Noctuoidea											
Erebidae											
<i>Paragabara curvicornuta</i>	15,532	80.5	0.009	-0.224	3722	78.7	2274	85.1	340	91.5	NC034638.1
<i>Amata formosae</i>	15,463	79.5	-0.026	-0.268	3730	77.8	2163	83.7	482	92.7	NC021416.1
Noctuidae											
<i>Agrotis segetum</i>	15,378	80.7	0.004	-0.192	3727	79.1	2164	85.1	332	94.3	KC894725.1
<i>Ctenoplusia agnate</i>	15,261	81.1	-0.023	-0.185	3735	79.9	2112	84.6	334	93.4	KC414791.1
<i>Helicoverpa assulta</i>	15,400	81	-0.005	-0.2	3725	79.5	2222	85.3	330	94.8	KT626655.1
Notodontidae											
<i>Clostera anachoreta</i>	15,456	80.7	-0.019	-0.212	3718	79.2	2259	85.2	347	91.9	NC034740.1
Nolidae											
<i>Risoba prominens</i>	15,343	81	-0.007	-0.175	3727	79.6	2203	85.5	342	93.6	NC026841.1
Bombycoidea											
Bombycidae											
<i>Bombyx mandarina</i>	15,717	81.4	-0.057	-0.215	3714	79.6	2168	85	495	95.5	FJ384796.1
<i>Triuncina daii</i>	15,482	81.6	0.002	-0.222	3733	80.1	2230	85.4	361	94.2	NC036484.1
Saturniidae											
<i>Antheraea yamamai</i>	15,338	80.3	-0.021	-0.218	3729	78.9	2156	84.2	334	89.5	EU726630.1
<i>Saturnia boisduvalii</i>	15,360	80.6	-0.025	-0.216	3734	79.2	2165	84.5	330	91.5	EF622227.1
Endromidae											
<i>Andraca theae</i>	15,737	78.3	0.029	-0.281	3723	76	2157	83.5	552	88.6	NC032694.1
Sphingidae											
<i>Ampelophaga rubiginosa</i>	15,282	81.5	0.016	-0.189	3724	80.5	2119	84.7	399	92.2	NC035431.1
<i>Manduca sexta</i>	15,516	81.8	-0.005	-0.18	3718	80.3	2168	85.4	324	95.4	EU286785.1
Lasiocampoidea											
Lasiocampidae											
<i>Kunugia undans</i>	15,570	78.6	-0.036	-0.277	3735	76.6	2296	84.3	317	88.7	NC035683.1
<i>Trabala vishnou</i>	15,281	80.9	-0.004	-0.208	3726	79.4	2132	85.2	346	91.7	KU884483.1
<i>Dendrolimus spectabilis</i>	15,409	79.4	-0.028	-0.223	3724	77.7	2231	84.4	320	92.5	KU558688.1

Table 1 (continued)

Taxon	Mitogenome (majority strand)				PCG ^b		rRNA		AT-rich region		GenBank accession No.
	Size (bp)	AT (%)	AT-skew	GC-skew	NO codons ^a	AT (%)	Size (bp)	AT (%)	Size (bp)	AT (%)	
Geometroidea											
Geometridae											
<i>Jankowskia athlete</i>	15,534	79.5	-0.042	-0.22	3728	77.7	2171	83.8	675	91.4	NC027948.1
<i>Apocheima cinerarium</i>	15,722	80.8	-0.027	-0.188	3732	77.8	2179	85.2	625	95.8	NC024824.1
<i>Biston panterinaria</i>	15,516	79.5	0.064	-0.259	3727	77.4	2261	85.2	349	93.1	NC020004.1
Pyraloidea											
Crambidae											
<i>Parapoynx crisonalis</i>	15,374	82	-0.017	-0.156	3723	80.5	2172	85.6	422	96.4	NC031151.1
Papilionoidea											
Papilionidae											
<i>Lamproptera curius</i>	15,277	80.5	-0.009	-0.194	3719	79.3	2119	83.6	470	89.8	KJ141168.1
<i>Parnassius bremeri</i>	15,389	81.3	-0.011	-0.191	3721	80.2	2117	84.4	504	93.6	NC014053.1
<i>Parnassius epaphus</i>	15,458	81.4	-0.017	-0.194	3723	79.9	2119	84.6	496	91.8	KM373898.1
<i>Papilio polytes</i>	15,256	81.1	-0.023	-0.206	3731	79.9	2143	84.3	428	95.1	NC024742
Nymphalidae											
<i>Hipparchia autonoe</i>	15,489	79.1	-0.016	-0.244	3722	76.9	2110	84.3	678	94.6	GQ868707.1
<i>Heliconius clysonymu</i>	15,302	79.7	-0.034	-0.202	3715	78.7	2168	83.1	427	83.1	NC027516.1
<i>Apatura ilia</i>	15,242	80.5	-0.011	-0.204	3710	78.9	2109	85	403	92.5	NC016062.1
<i>Idea leuconoe</i>	15,277	80.4	-0.027	-0.231	3709	78.9	2130	84.4	444	94.8	NC030376.1
Riodinidae											
<i>Abisara fylloides</i>	15,301	81.2	-0.027	-0.202	3459	79.8	2105	85.5	423	91	HQ259069.1
Pieridae											
<i>Delias hyparete</i>	15,186	79.8	-0.175	-0.228	3714	78.5	2110	84.2	377	92	NC020428.1
Lycaenidae											
<i>Spindasis takanonis</i>	15,349	82.3	0.004	-0.216	3730	81.1	2110	85.2	371	94.6	HQ184266.1
<i>Coreana raphaelis</i>	15,314	82.7	-0.047	-0.156	3717	81.5	2107	85.5	375	94.2	DQ102703.1
Hesperoidea											
Hesperiidae											
<i>Euschemon rafflesia</i>	15,447	81.4	-0.039	-0.204	3718	79.9	2126	84.7	453	95.3	NC034231.1
<i>Achalarus lyciades</i>	15,612	81.9	-0.011	-0.193	3726	80	2156	85.6	452	95.1	NC030602.1

Table 1 (continued)

Taxon	Mitogenome (majority strand)				PCG*		rRNA		AT-rich region		GenBank accession No.
	Size (bp)	AT (%)	AT-skew	GC-skew	NO codons**	AT (%)	Size (bp)	AT (%)	Size (bp)	AT (%)	
Tortricioidea											
Tortricidae											
<i>Retinia pseudotsugaicola</i>	15,282	79.8	-0.005	-0.223	3713	78.2	2151	83.9	358	97.2	NC022865.1
<i>Grapholita molesta</i>	15,716	80.9	-0.003	-0.173	3740	78.9	2149	85	770	96	HQ392511.1
<i>Spilonota lechriaspis</i>	15,368	81.2	-0.017	-0.191	3741	79.7	2160	85.5	441	92.8	HM204705.1
<i>Lobesia botrana</i>	15,229	80.1	0.001	-0.206	3707	78.5	2149	84.8	286	91.6	NC029193.1
<i>Cydia pomonella</i>	15,253	80.1	-0.004	-0.206	3725	78.5	2147	84.4	351	95.7	JX407107.2
<i>Rhyacionia leptotubula</i>	15,876	80.3	-0.001	-0.228	3571	78.4	2133	83.8	739	95.3	NC019619.1
Drepanoidea											
Drepanidae											
<i>Oreta fuscopurpurea</i>	15,565	79.6	0.015	-0.206	3733	77.8	2187	83.7	527	93.0	MG572766
<i>Drepana arcuate</i> ***	—	—	—	—	3704	79.7	—	—	—	—	KJ508053.1
Doidae											
<i>Doa</i> sp.***	—	—	—	—	3702	79.1	—	—	—	—	KJ508058.1

*Protein-coding genes.

**Termination codons were excluded in total codon count.

***Incomplete mitochondrion gene, the dash means the gene was not detected.

Table 2. Basic components of the *O. fuscopurpurea* mitogenome.

Gene	Direction	Location	Size (bp)	Intergenic length*	Anticodon	Start codon	Stop codon
tRNA ^{Met}	F	1–68	68	1	CAT	-	-
tRNA ^{Ile}	F	70–133	64	-3	GAT	-	-
tRNA ^{Gln}	R	131–199	69	58	TTG	-	-
ND2	F	258–1271	1014	-1	-	ATT	TAA
tRNA ^{Trp}	F	1271–1337	67	-8	TCA	-	-
tRNA ^{Cys}	R	1330–1397	68	3	GCA	-	-
tRNA ^{Tyr}	R	1401–1465	65	5	GTA	-	-
COI	F	1471–3001	1531	0	-	CGA	T-tRNA
tRNA ^{Leu} (UUR)	F	3002–3068	67	0	TAA	-	-
COII	F	3069–3750	680	0	-	ATG	T-tRNA
tRNA ^{Lys}	F	3751–3821	71	2	CTT	-	-
tRNA ^{Asp}	F	3824–3890	67	0	GTC	-	-
ATP8	F	3891–4052	162	-7	-	ATC	TAA
ATP6	F	4046–4723	678	3	-	ATG	TAA
COIII	F	4727–5518	790	4	-	ATG	TAA
tRNA ^{Gly}	F	5521–5587	67	0	TCC	-	-
ND3	F	5588–5941	354	20	-	ATT	TAA
tRNA ^{Ala}	F	5962–6027	66	-1	TGC	-	-
tRNA ^{Arg}	F	6027–6089	63	0	TCG	-	-
tRNA ^{Asn}	F	6090–6155	66	4	GTT	-	-
tRNA ^{Ser} (AGN)	F	6160–6223	64	11	GCT	-	-
tRNA ^{Glu}	F	6235–6300	66	9	TTC	-	-
tRNA ^{Phe}	R	6310–6376	67	6	GAA	-	-
ND5	R	6383–8123	1741	0	-	ATT	T-tRNA
tRNA ^{His}	R	8124–8188	65	0	GTG	-	-
ND4	R	8189–9527	1339	-1	-	ATG	T
ND4L	R	9527–9817	291	4	-	ATG	TAA
tRNA ^{Thr}	F	9822–9886	65	0	TGT	-	-
tRNA ^{Pro}	R	9887–9952	66	2	TGG	-	-
ND6	F	9955–10482	528	4	-	ATA	TAA
Cytb	F	10486–11658	1173	6	-	ATG	TAA
tRNA ^{Ser} (UCN)	F	11665–11729	65	13	TGA	-	-
ND1	R	11743–12686	944	-1	-	ATA	TA
tRNA ^{Leu} (CUN)	R	12686–12756	71	-3	TAA	-	-
12S rRNA	R	12754–14162	1409	6	-	-	-
tRNA ^{Val}	R	14169–14235	67	23	TAC	-	-
srRNA	R	14259–15036	778	2	-	-	-
AT-rich region		15039–15564	526	0	-	-	-

*The negative indicates the overlapping base pairs, and the positive indicates the interval base pairs.

3.5 The A+T rich region

The A+T rich region (control region) is 526 bp, located between *srRNA* and tRNA^{Met} (Figs 1, 5). Its AT content is 92.9% fallen within the range from 83.1% in *Heliconius clysonymu* to 97.2% in *Retinia pseudotsugaicola* (Table 1). The *O. fuscopurpurea* A+T rich region also possessed several conserved structures common to lepidopterans, such as the O_N (the origin of gene replication) harboring the motif ATAGA which is located 16 bp downstream of the *SrRNA* gene and followed by an 19 bp poly-T stretch, the poly-A, poly-T, (TA)₁₀, (AT)₈, and two 18 bp tandem repetitive sequences TATAATAATATA TATATA; in addition, an extra tRNA^{Asn}-like structure is detected in the region, and the similar cases are also found in other previously determined lepidopterans (Zhang *et al.*, 1995; Schulthe *et al.*, 2002; Pan *et al.*, 2008; Wang *et al.*, 2015) (Fig. 5).

3.6 Phylogenetic analysis

The superfamily Drepanoidea was once placed in the Geometroidea for their synapomorphic abdominal tympanal organs (Imms, 1934) and composed of two families, the Drepanidae and the Epicopeiidae (Minet, 1991) and thus the

classification system of Drepanoidea by Minet has been widely accepted and used (Holloway, 1998; Holloway *et al.*, 2001; Kristensen *et al.*, 2007; Cho *et al.*, 2011). However, some scholars suggested that the Drepanoidea should exclude the family Epicopeiidae in morphological view (Kuznetsov & Stekolnikov, 2001; Wu *et al.*, 2009); Moreover, Regier *et al.* (2009) and Mutanen *et al.* (2010) revealed that the traditional Drepanoidea should be divided into two independent groups, that is the Epicopeiidae was sister to the superfamily Geometroidea; while the Drepanidae was sister to the grouping of (Cimeliidae + Doidae) by molecular and morphological criteria; afterwards, Nieuwerkerken *et al.* (2011) proposed that the superfamily Drepanoidea consisted of three families, Drepanidae, Cimeliidae and Doidae, and this view was widely accepted later by most scholars (Bazinet *et al.*, 2013; Heikkilä *et al.*, 2015), though it was incongruent with that upon 19 nuclear protein-coding genes (Regier *et al.*, 2013) in which the Drepanoidea included another family Mimallonidae.

The maximum likelihood (ML) and Bayesian (BI) trees reconstructed of this study were absolutely the same in topology, with a relatively strong supports of each node in the bootstrapping and posterior probability (Fig. 6). Both of the two trees distinctly showed that (1) the phylogenetic relationships within the main lepidopteran groups of this study is (Tortricoidea +

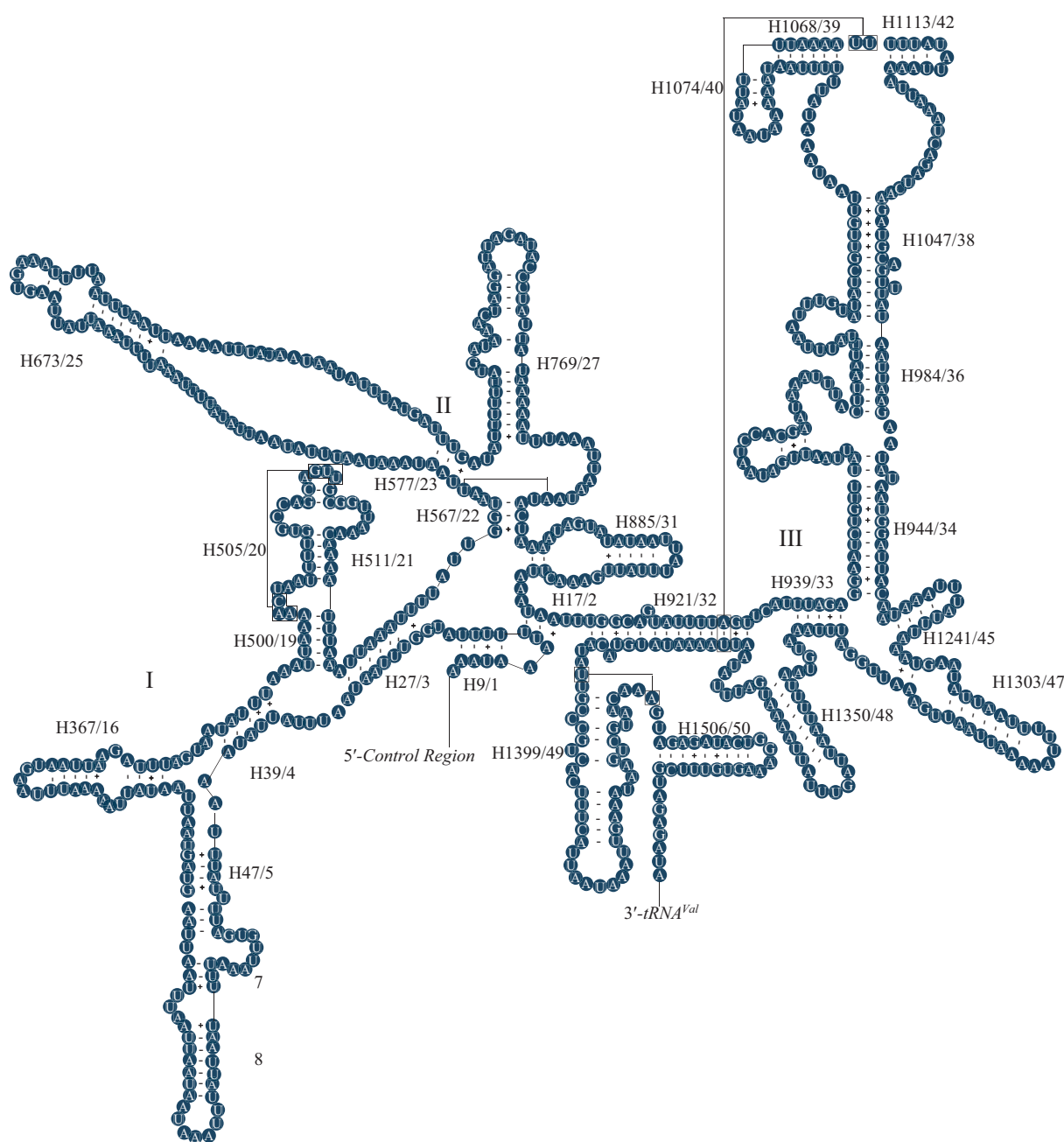


Figure 4. Predicted secondary structure of the *O. fuscopurpurea* srRNA. The annotation is the same as in Figure 3.

(Pyraloidea + (((Noctuoidea + Geometroidea) + (Bombycoidea + Lasiocampoidea)) + Drepanoidea)); (2) the superfamily of Drepanoidea represented hereby three species (*O. fuscopurpurea*, *Drepana arcuata* and *Doa* sp.) is sister to the grouping of ((Bombycoidea + Lasiocampoidea) + (Noctuoidea + Geometroidea)), with a relatively strong support for BI tree (posterior probability value 1.00) and ML tree (bootstrap value 100%).

As for the phylogenetic relationships with the main lepidopteran groups of this study, our analysis showed that (1) the superfamily Pyraloidea was more closely related to the five macrolepidopteran superfamilies (Bombycoidea, Geometroidea, Noctuoidea, Drepanoidea, Lasiocampoidea) than the macrolepidopteran superfamily Papilionoidea; (2) the Geometroidea and Noctuoidea made up a clade, while the Bombycoidea and Lasiocampoidea constituted another, then the two clades were grouped with the Drepanoidea, which is consistent with the results of Liao *et al.* (2010) and Chai *et al.* (2012), but contradictory to the result of Yang *et al.* (2013); (3) the Hesperioidea was nested within the Papilionoidea, and thus all the butterfly groups is monophyletic, which is in accordance with some previous studies (Wei *et al.*, 2013; Regier *et al.*, 2013; He *et al.*, 2015), however, somewhat different with our previous studies (Hao *et al.*, 2012). (4) Doidae (*Doa* sp.) belonging to the Drepanoidea was consistent with the previous results of van Nieukerken *et al.* (2011) and Bazinet *et al.* (2013), strengthening its separation from Noctuoidea (Miller, 1991).

A *O. fuscopurpurea* AT-rich region

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srRNA -ATATAAATAAATTTGCATAGATTTTTTTTTTTTTTTTTTAAATATTAATAATTTA
                                poly-T
    ATAGAAATTATTAACATTTAATAATTTTTTTTTTTTTTTTTTCATAACATTAAT
                                poly-T
    ATTGAAAATTGCATTTAATTATAAATCAATATATAATCATTAAATAATAATATA
    TTAAGATAATTAATTTTAATTTTTTAAAGTTAGTTATTATATTAATATATATAATAT
                                Tandem repeats
    AATAATATATATATATATATATTTTAATTTATAATTATTTAAATTATTTAATTTAA
    (AT)8 ← tRNAAsn-like sequence
    AAAAAAAAAATAATATTATTTTATATAATTACATAAATATATGTATATATATATAT
    poly-A
    ATATATATTTATAAACTTTATATAATAATATTTCTATATATTTAAATATATACTTAA
    (TA)10
    ATAAAAATAAAAAATAAATAAATACATACATACACCACAAATAAAATAAGAAG
    AAAAATAAGTATAAATTAACTTAACCATACCTAATAAAAAATACAATAATAATAA
    TAAATAATAACGATAATT -tRNAMet
  
```

B tRNA-like sequences

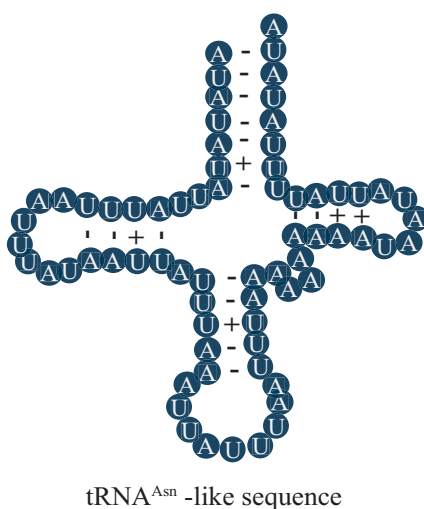


Figure 5. AT-rich region of the *O. fuscopurpurea* mitogenome. A. Sequence of *O. fuscopurpurea* AT-rich region. The shadow areas show the poly-T stretch, poly-A stretch, microsatellite (AT)₈ and (TA)₁₀ repeat sequence. The underlined sequences show the tRNA^{Asn}-like sequence. B. Secondary structure of the tRNA^{Asn}-like sequence. Watson-Crick pairs are joined by minus signs (-), other interactions are joined by plus signs (+).

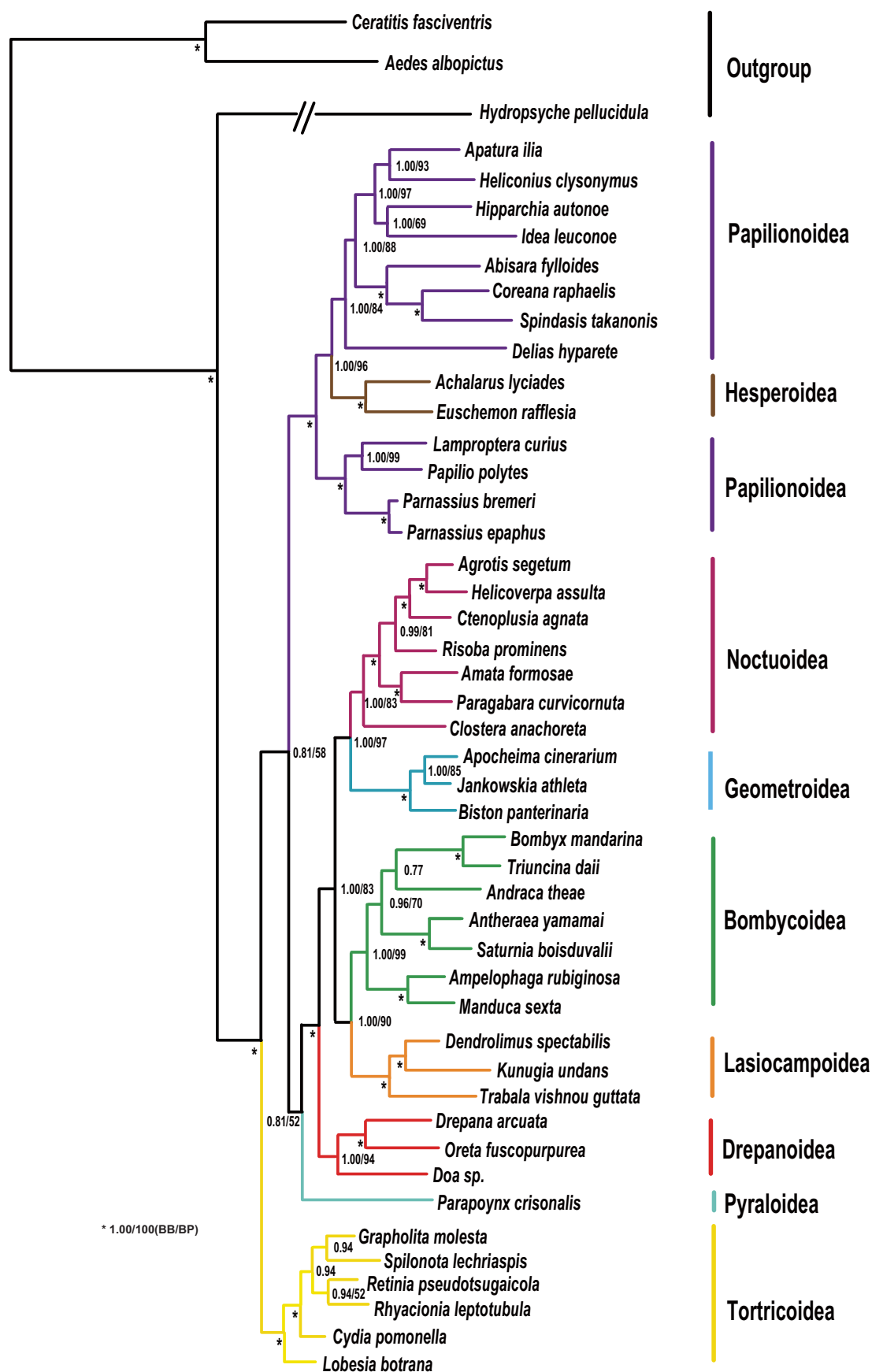


Figure 6. The Bayesian inference and maximum likelihood phylogenetic trees among 44 lepidopteran species in this study (the numbers on each node represent the BB value of the BI tree and BP value of the ML tree). The value of BP lower than 50 or the value of BB lower than 0.5 were not shown on the picture, asterisk represents the BP value of 100 and the BB value of 1.00.

Our results distinctly indicated that the Drepanoidea (represented only by *O. fuscopurpurea*, *D. arcuata* and *Doa sp.* in this study) was closely related to the clade of ((Bombycoidea + Lasiocampoidea) + (Noctuoidea + Geometroidea)). As far as we know, some previous morphological studies suggested that they were probably sister to the Geometroidea in that both of them shared some synapomorphic characters, such as the strong anterior extension of pupal forelegs, the presence of a transverse dorsal groove on pupal segment A10, the abdominal tympanal organs and so on (Kristensen *et al.*, 2003), though a few studies revealed that their tympanal organs are significantly different and evolved independently, their tegula ventral arms are also remarkably different in appearance (Hasenfuss, 2000; Surlykke *et al.*, 2003; Heikkilä *et al.*, 2015). All these uncertainties are still awaiting further detailed phylogenetic investigations by using broader sampling and more molecular and morphological data.

Table 3. Relative synonymous codon usage (RSCU) and the codons per thousand codons (CDspT) in *O. fuscopurpurea* PCGs.*

Amino acid	Codon	RSCU	CDspT	Amino acid	Codon	RSCU	CDspT
F	TTT	1.75	90.94	S2	TCT	2.78	29.62
	TTC	0.25	13.24		TCC	0.33	3.48
L	TTA	4.92	122.3		TCA	1.74	18.47
	TTG	0.17	4.18		TCG	0.15	1.74
L2	CTT	0.44	11.15	P	CCT	2.28	18.47
	CTC	0.08	1.74		CCC	0.26	2.09
	CTA	0.37	9.06		CCA	1.45	11.85
	CTG	0.02	0.7		CCG	0	0.00
I	ATT	1.84	110.8	T	ACT	2.06	21.6
	ATC	0.16	9.41		ACC	0.36	3.83
M	ATA	1.77	64.46		ACA	1.5	15.68
	ATG	0.23	8.71		ACG	0.08	0.7
V	GTT	1.88	18.82	A	GCT	2.14	19.16
	GTC	0.16	1.74		GCC	0.33	2.79
	GTA	1.8	18.12		GCA	1.41	12.54
	GTG	0.16	1.74		GCG	0.12	1.05
Amino acid	Codon	RSCU	CDspT	Amino acid	Codon	RSCU	CDspT
Y	TAT	1.6	39.02	C	TGT	1.66	7.67
	TAC	0.4	9.76		TGC	0.34	1.74
		0	0	W	TGA	1.87	23.69
		0	0		TGG	0.13	1.74
H	CAT	1.65	15.33	R	CGT	0.98	3.14
	CAC	0.35	3.14		CGC	0	0.00
Q	CAA	1.88	17.42		CGA	2.78	9.06
	CAG	0.12	1.05		CGG	0.24	0.7
N	AAT	1.82	63.41	S	AGT	0.98	10.45
	AAC	0.18	6.27		AGC	0.1	1.05
K	AAA	1.85	26.13		AGA	1.89	20.21
	AAG	0.15	2.09		AGG	0.03	0.35
D	GAT	1.73	13.94	G	GGT	0.77	9.76
	GAC	0.27	2.09		GGC	0.15	1.74
E	GAA	1.68	16.72		GGA	2.16	27.87
	GAG	0.32	3.14		GGG	0.93	12.2

*Termination codons were excluded to the count due to the uncertainty in many species.

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