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Characterization of the Complete Mitochondrial Genome of *Salassa thespis* (Lepidoptera: Saturniidae) and Comparison with Other Lepidoptera Species

Shengni Tian [1,†], Yongqi Zhang [1,†], Xiangrong Meng [1], Yuan Liu [1], Xin Dong [1] and Cen Qian [1,2,*]

[1] College of Life Sciences, Anhui Agricultural University, 130 ChangJiang West Road, Hefei 230036, China; tiansn@ahau.edu.cn (S.T.); 15056582113@163.com (Y.Z.); 15656060932@163.com (X.M.); liuyuan202405@163.com (Y.L.); 13083053602@163.com (X.D.)

[2] Anhui Province Key Laboratory of Resource Insect Biology and Innovative Utilization, School of Life Sciences, Anhui Agricultural University, Hefei 230036, China

* Correspondence: qiancenqiancen@163.com or qiancen@ahau.edu.cn

† These authors contributed equally to this work.

Abstract: There are many species of Lepidoptera, but few complete mitochondrial genomes of Lepidoptera have been included in databases. Here, the complete mitochondrial genome sequence of *Salassa thespis* was isolated and characterized. It was 15,302 bp in length and contained 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes and an A + T-rich region. Among the 13 PCGs, the initiation codon of *cytochrome c oxidase subunit 1* (*cox1*) was CGA, and the rest were ATN. The *cox1* and *cox2* genes had an incomplete stop codon T, while the rest terminated with TAA. Codon usage analysis showed that Phe, Ile, Leu and Asn were the most frequent amino acids, while Trp was the least. Like other Lepidopterans, some conserved motifs were found in the A + T-rich region, including a 17 bp poly-T guided by ATAGA, the AT-rich area and a poly-A element. Bayesian inference and maximum likelihood phylogenetic tree analysis based on 13 PCGs of *S. thespis* confirmed that it belonged to the Saturniidae family and showed the following relationship: (*S. thespis* + (*Rhodinia fugax* + *Samia canningi*)). The enrichment of mitochondrial DNA provides reference information for the study of the evolution and diversity of Lepidoptera insects.

Keywords: *Salassa thespis*; mitochondrial genome; Lepidoptera; phylogeny

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1. Introduction

The insect mitochondrial DNA (mtDNA) is usually a compact double-stranded closed circular molecule with a length of 14–20 kb. It is composed of 13 protein-coding genes (PCGs), two rRNA genes (12S rRNA and 16S rRNA), 22 tRNA genes and one or more noncoding regions containing transcription and replication signals [1]. Insect mtDNA is characterized by a simple structure, stable composition, rapid evolution, high copy number and low maternal inheritance and recombination rate. Compared with a single gene or a few genes, the mitochondrial genome has significant advantages in the study of insect species evolution, phylogeny, population genetics and diversity [2–5]. It can also be used to explore intraspecific relationships, including population genetic differentiation and migration diffusion, such as how *Bombyx mori* was domesticated and the diffusion pathway of *Bombyx mandarina* which have been the focus of research in recent years. Chen et al. discussed the genetic relationship between *B. mori* and *B. mandarina* in different geographical populations from the perspective of the mitochondrial genome [6].

Lepidoptera is the second largest order in Insecta, mainly composed of moths and butterflies [7]. Nearly 160,000 species of Lepidoptera have been described worldwide, which are important agricultural and forestry pests, pollinators, economic insects and ornamental insects. Initially, the mitochondrial genome was widely used in Lepidoptera to

solve the species identification controversy left over from the traditional morphological classification, and *Bombyx mori* was the first reported species. Subsequently, research on mitochondrial genomes covered various levels and groups of Lepidoptera insects, especially among species at different superfamily and family levels, and was used to explore the origin, divergence, diffusion and phylogeny of species [8–13]. However, the complete mitochondrial genome sequences of Lepidoptera insects included in the GenBank database account for less than 1% of the total number of Lepidoptera.

Salassa thespis (Leech, 1898) is mainly distributed in Shaanxi, Fujian, Hubei, Sichuan and other regions of China. *S. thespis* has a larger body size, with wings extending 11–12 cm. Its body is brownish red to yellow brown, and its hind wings resemble the eyes of a cat, making it popular for its ornamental value; however, its mitochondrial genome has not been described and analyzed.

Here, the mitochondrial genome of *S. thespis* was sequenced and its characteristics were analyzed, including the length of mtDNA sequence, the content of four bases (A, T, C and G), mitochondrial protein-coding genes, rRNA and tRNA genes, the A + T-rich region, codon usage preferences, etc. A comparison was made with the mitochondrial genomes of other reported Lepidoptera insects, and a phylogenetic tree was constructed by Bayesian inference (BI) and maximum likelihood (ML) methods. This study enriches the information of the mitochondrial genome of Lepidoptera and provides a reference for further exploring the phylogeny, classification, evolution and diversity of Lepidoptera species.

2. Materials and Methods

2.1. Experimental Insects and DNA Extraction

S. thespis adults were collected from Niba Mountain, Ya'an City, Sichuan Province, China, and stored in a refrigerator at −80 °C. Total genomic DNA was extracted by the Aidlab Genomic DNA Extraction Kit (Aidlab Co., Beijing, China). DNA quality was determined by 1% agarose gel electrophoresis, and the whole mitochondrial genome of *S. thespis* was amplified.

2.2. PCR Amplification and DNA Sequencing

Mitochondrial DNA fragments were amplified by polymerase chain reaction (PCR) using total genomic DNA as the template. Fourteen pairs of primers were designed and synthesized by General Biosystems Co., Chuzhou, China, to amplify the mitochondrial genome of *S. thespis* (Table 1). PCR amplifications were performed on a 20 μL reaction volume consisting of 7 μL sterile distilled water, 1 μL extracted DNA as the template, 1 μL each of forward and reverse primers and 10 μL (1 unit) Taq DNA polymerase (Takara Co., Dalian, China). PCR amplification conditions were as follows: first denaturation at 94 °C for 5 min, followed by 30 cycles (94 °C, 30 s; 50–55 °C, 1 min; 72 °C, 1–2 min) and finally 72 °C, 10 min for subsequent extension. The PCR products were separated by 1% agarose gel electrophoresis and purified using a DNA gel extraction kit (TransGen Co., Beijing, China) and sent to General Biosystems Co., Chuzhou, China, for sequencing at least 3 times.

Table 1. The primers used to amplify the mitogenome of *S. thespis*.

Primer Names	Primer Sequence (5′→3′)
F1	TGACAAAAAATTACCCCCATAA
R1	GCTCCATTTTCTACAATTCTTC
F2	TGATTAGTTCCTTTAATATTAGG
R2	TGTTCTATTAATGGAGATGCC
F3	GTAATGGATTTAAACCCCATA
R3	AATATATGTTGATAATTATTAATTC
F4	TATCTCTCTCTATTTCTTTACC
R4	ATAGAAAGGAATATTATAATATTAG

Table 1. *Cont.*

Primer Names	Primer Sequence (5′→3′)
F5	TATATAATATATTTAGTATATTTGA
R5	TTTATTTATTTAGATTTATTTTATG
F6	CAATATTATGCTCTATATAAGC
R6	AGTTCTTGATTACCAGCTGC
F7	TATATTAAATCGAATTAATAAATA
R7	CTATATATTTTGTTCATTTATGA
F8	CCTAATAATCTAATTCTCACTC
R8	ATTCTTTCTTTGAAGTTTTTAAAG
F9	ATTGACCCTGAAACTGGAGC
R9	GAGTTAGGGTAGCATTATCTAC
F10	TTGAATTTGAGGGGGATTTGC
R10	GATAAATTAATGTATTTGGCTTG
F11	GCATTTGTTTTGAAAACTTAAGAA
R11	CATAAATAAGTGAATAAATGATCC
F12	GTAAGATTTTAATGATCGAACAG
R12	CAAAGTAGAGGTACTGGAAAG
F13	TTACAATACTAATTAACTATAAAC
R13	TGAGGTATGAGCCCAAAAGC
F14	CCATAAGAAATTTATTTATTCATG
R14	AATTATTGATTGAGGAGAAAGC

2.3. Sequences Assembly, Annotation and Analysis

We checked the sequence obtained by sequencing in 2.2 with the original peak map of sequencing to prevent inaccurate results due to base site changes caused by software recognition errors or splicing errors. The blast tool and Lasergene v7.1.0 provided by NCBI (http://blast.ncbi.nlm.nih.gov/Blast, accessed on 20 September 2023) were used to remove the cloning vector sequence, delete the fragment repeat sequence and assemble the sequence. Mitochondrial genomes of related species in GenBank were aligned and retrieved, and the position, start codon and stop codon of each gene were corrected. The final length of the mitochondrial sequence was determined by sequence assembly and gene annotation. Preliminary gene predictions and annotations were carried out using the Mitos web server (specific parameter settings: 05-inverterbrate codon table; the RefSeq lineage was 89-Metazoa; and the rest were set according to Mitos default parameters) [14]. The mitochondrial genome map was constructed using the Proksee server [15]. According to the coding principle of invertebrate mitochondrial genomes, the base sequence of the coding protein was translated into a predicted protein. The tRNA and rRNA geneswere amplified by the online software tRNAscan-SE v2.0 (http://lowelab.ucsc.edu/tRNAscan-SE/, accessed on 2 October 2023) [16] and MITOS web server v1.1.7 (http://mitos.bioinf.uni-leipzig.de/index.py, accessed on 2 October 2023), using Gblocks v0.91b to remove ambiguously aligned sites, and every tRNA and rRNA gene was aligned on the MAFFT v7 server [17,18], using Gblocks to prune the poorly aligned regions. The annotated sequences were submitted to GenBank of NCBI (accession number: OR522707). The base content of A, T, C and G and the relative synonymous codon usage (RSCU) of mitochondrial genomes were calculated by MEGA 6.0 [19]. The calculation of base skew was based on the following formula: AT skewness = (A − T)/(A + T); GC skewness = (G − C)/(G + C) [20].

2.4. Phylogenetic Analysis

Thirty-three complete mitochondrial genome sequences were downloaded from the GenBank database to construct the phylogenetic tree, in which *Drosophila melanogaster* (accession number: U37541) and *Anopheles gambiae* (accession number: L20934) were used as outgroups (Table 2). Multiple alignments of PCGs were performed using Clustal X version 2.0 [21]. We used the MAFFT algorithm to perform a translation alignment on each PCG separately, and then we performed a single alignment on each sample. We

used Gblocks to remove areas with poor alignment and alignment gaps. Based on 13 PCGs, Bayesian inference (BI) and maximum likelihood (ML) methods were used to construct phylogenetic trees [5]. BI with MrBayes 3.2 version [22] was used under the GTR + I + G nucleotide model selected with PartitionFinder 2 [23]. The Markov chains were run with at least one million generations. When the convergence diagnostic value was less than 0. 01, the operation ended. ML with IQ-TREE v1.6.12 [24] was used under the GTR + I + G model selected with ModelFinder [25], and branch supports were assessed using 1000 bootstrap replicates. The software FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 5 October 2023) was used to view and edit the phylogenetic tree.

Table 2. List of sequences used to construct the phylogenetic tree.

Superfamily	Family	Species	Length (bp)	Accession Number
Bombycoidea	Saturniidae	*Salassa thespis*	15,301	OR522707.1
		Rhodinia fugax	15,334	NC_059700
		Samia canningi	15,384	NC_024270
	Sphingidae	*Parum colligata*	15,288	NC_039166
		Sphinx morio	15,299	NC_020780
	Lasiocampidae	*Euthrix laeta*	15,368	NC_031507
Noctuoidea	Noctuidae	*Sphragifera sigillata*	15,368	NC_061640
		Athetis pallidipennis	15,344	NC_046525
	Euteliidae	*Eutelia adulatricoides*	15,360	NC_026840
	Nolidae	*Pseudoips prasinana*	15,239	NC_062184
		Eligma narcissus	15,346	NC_062104
	Notodontidae	*Phalera grotei*	15,397	OQ830676
Drepanoidea	Drepanidae	*Tethea albicostata*	15,308	NC_061643
		Pseudalbara parvula	15,304	NC_065769
Geometroidea	Uraniidae	*Lyssa zampa*	15,571	MZ713634
	Geometridae	*Scopula subpunctaria*	15,464	NC_067763
Pyraloidea	Crambidae	*Herpetogramma luctuosalis*	15,342	OQ472987
		Marasmia exigua	15,262	MN877384
		Cydalima perspectalis	15,232	NC_042150
Gelechioidea	Gelechiidae	*Pectinophora gossypiella*	15,202	NC_065403
		Dichomeris ustalella	15,410	NC_029810
	Scythrididae	*Scythris sinensis*	15,216	NC_048471
Papilionoidea	Nymphalidae	*Libythea lepita*	15,167	NC_080335
		Issoria eugenia	15,206	NC_050261
	Papilionidae	*Parnassius apollonius*	15,245	OP881960
Cossoidea	Cossidae	*Endoxyla cinereus*	15,285	NC_062621
Hyblaeoidea	Hyblaeidae	*Hyblaea puera*	15,350	MW885970
Tortricoidea	Tortricidae	*Grapholita dimorpha*	15,813	NC_024582
		Grapholita molesta	15,776	HQ116416
Copromorphoidea	Carposinidae	*Carposina sasakii*	15,611	NC_023212
Hesperioidea	Hesperiidae	*Coladenia maeniata*	15,284	NC_079683

3. Results

3.1. Genome Structure and Characteristics

The complete mitogenome of *S. thespis* was a circular double-stranded DNA molecule with a total length of 15, 302 bp (Figure 1). It contained 22 tRNA genes, 13 PCGs (*atp6*, *atp8*, *cob*, *cox1–cox3*, *nad1–nad 6* and *nad 4L*), two rRNA genes (*rrnL* and *rrnS*) and an A + T-rich region (details are reported in Table 3. The heavy chain (H-chain) of *S. thespis* encoded 23 genes (nine PCGs and 14 tRNAs), while the light chain (L-chain) encoded 14 genes (four PCGs, 8 tRNAs and two rRNAs). The differences in the nucleotide composition of mitochondrial genomes are usually reflected by AT content, AT skewness and GC skewness. Here, the nucleotide composition of the *S. thespis* mitogenome exhibited 78.8% AT content (A: 39.17%, T: 39.63%) and 21.2% GC content (G: 8.19%, C: 13.01%), and especially in the A + T-rich region, the AT content was as high as 90.33% (Table 4). The AT skewness of the whole genome of *S. thespis* was negative (−0.01), as found in *Eochroa trimenii*, *Sphragifera sigillata*, *Eutelia adulatricoides*, *Herpetogramma luctuosalis*, *Marasmia exigua*, *Pectinophora gossypiella*, *Parnassius apollonius*, *Grapholita dimorpha* and *Grapholita molesta*. The GC skew-

ness of the whole genome of *S. thespis* was also negative (−0.23). The negative AT skewness and GC skewness of the *S. thespis* mitochondrial genome indicated the occurrence of more Ts than As and more Cs than Gs.

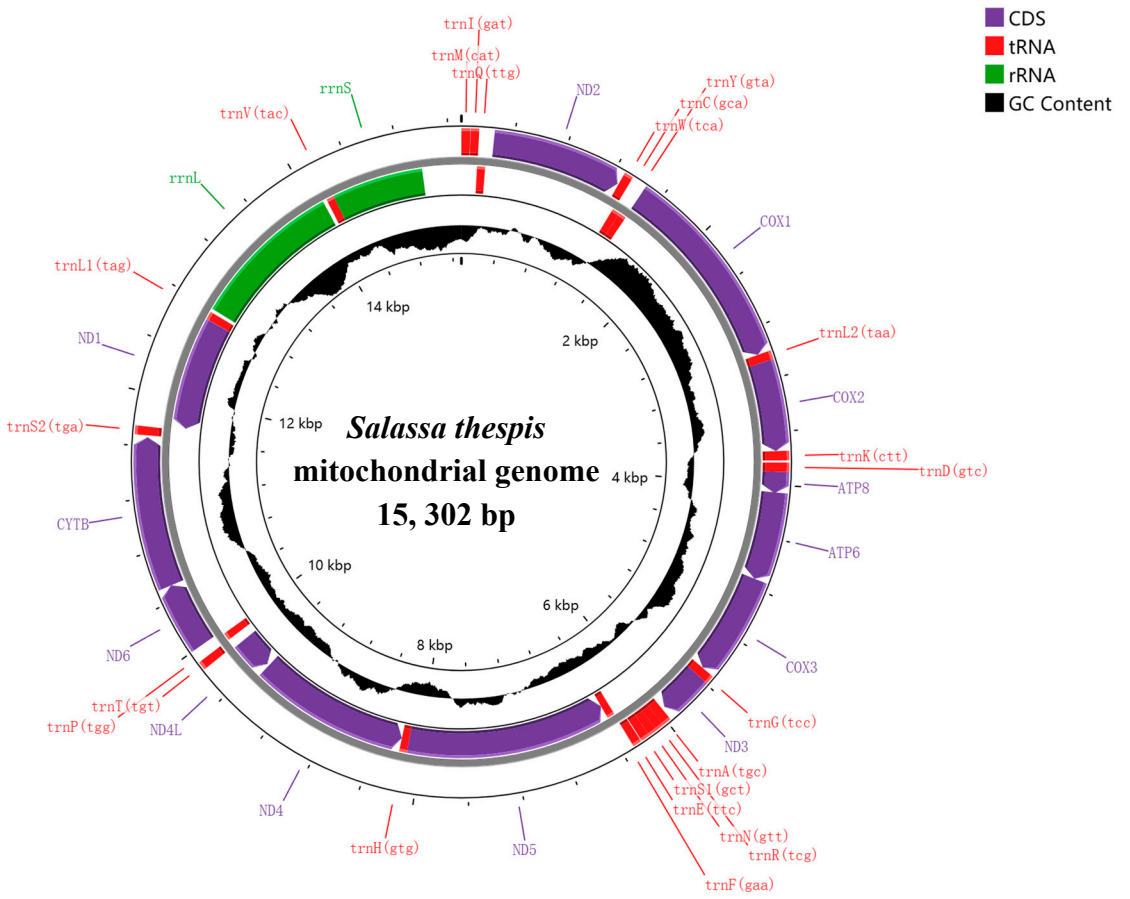

Figure 1. Map of the mitogenome of *S. thespis*.

Table 3. Characteristics of mitochondrial genome sequence of *S. thespis*.

Name	Start	Stop	Strand	Anticodon	Length	ovl/nc	Start and Stop Codons
trnM	1	67	+	CAT	67	2	/
trnI	70	134	+	GAT	65	−3	/
trnQ	132	200	-	TTG	69	50	/
nad2	251	1264	+	/	1014	8	ATT/TAA
trnW	1273	1341	+	TCA	69	−8	/
trnC	1334	1397	-	GCA	64	0	/
trnY	1398	1461	-	GTA	64	3	/
cox1	1465	2995	+	/	1531	0	CGA/T
trnL2	2996	3061	+	TAA	66	0	/
cox2	3062	3743	+	/	682	0	ATG/T
trnK	3744	3814	+	CTT	71	16	/
trnD	3831	3899	+	GTC	69	0	/
atp8	3900	4064	+	/	165	−7	ATC/TAA
atp6	4058	4735	+	/	678	−1	ATG/TAA
cox3	4735	5523	+	/	789	2	ATG/TAA
trnG	5526	5591	+	TCC	66	0	/
nad3	5592	5945	+	/	354	−7	ATT/TAA
trnA	5969	6037	+	TGC	69	−1	/

Table 3. *Cont.*

Name	Start	Stop	Strand	Anticodon	Length	ovl/nc	Start and Stop Codons
trnR	6037	6100	+	TCG	64	−1	/
trnN	6100	6165	+	GTT	66	0	/
trnS1	6166	6233	+	GCT	68	6	/
trnE	6240	6306	+	TTC	67	−2	/
trnF	6305	6368	-	GAA	64	−2	/
nad5	6367	8112	-	/	1746	0	ATA/TAA
trnH	8113	8177	-	GTG	65	0	/
nad4	8178	9518	-	/	1341	18	ATG/TAA
nad4l	9537	9827	-	/	291	16	ATG/TAA
trnT	9844	9908	+	TGT	65	0	/
trnP	9909	9972	-	TGG	64	6	/
nad6	9979	10,515	+	/	537	−1	ATC/TAA
cob	10,515	11,666	+	/	1152	14	ATG/TAA
trnS2	11,681	11,746	+	TGA	66	18	/
nad1	11,765	12,703	-	/	939	1	ATG/TAA
trnL1	12,705	12,772	-	TAG	68	29	/
rrnL	12,802	14,095	-	/	1294	33	/
trnV	14,129	14,195	-	TAC	67	0	/
rrnS	14,196	14,971	-	/	776	/	/
A + T-rich	14,972	15,302	/	/	331	/	/

Note: ovl/nc indicates the interval between the structure area and the previous structure area, and if it is a negative number, it indicates that the two areas overlap.

Table 4. Base composition and skewness in different mitogenomes of Lepidoptera.

Species	Size (bp)	A%	G%	T%	C%	A + T%	AT Skew	GC Skew
Whole genome								
S. thespis	15,302	39.17	8.19	39.63	13.01	78.80	−0.01	−0.23
E. trimenii	15,254	40.02	7.66	41.05	11.13	81.07	−0.01	−0.18
A. assama	15,312	39.35	7.71	40.82	12.11	80.17	−0.02	−0.22
P. colligata	15,288	40.74	7.65	40.31	11.29	81.05	0.01	−0.19
S. morio	15,299	40.64	7.58	40.53	11.26	81.17	0.00	−0.20
E. laeta	15,368	40.85	7.80	39.34	12.01	80.19	0.02	−0.21
S. sigillata	15,368	40.23	7.63	41.32	10.82	81.55	−0.01	−0.17
A. pallidipennis	15,344	39.53	7.69	41.47	11.31	81.00	−0.02	−0.19
P. prasinana	15,239	39.44	7.93	41.31	11.31	80.75	−0.02	−0.18
E. adulatricoides	15,360	40.21	7.81	40.66	11.33	80.87	−0.01	−0.18
E. narcissus	15,346	40.87	7.68	39.99	11.46	80.86	0.01	−0.20
P. grotei	15,397	40.48	7.65	40.04	11.83	80.52	0.01	−0.21
T. albicostata	15,308	40.27	7.87	40.25	11.61	80.52	0.00	−0.19
P. parvula	15,304	40.63	7.91	40.07	11.39	80.70	0.01	−0.18
L. zampa	15,571	41.55	7.31	40.18	10.96	81.73	0.02	−0.20
S. subpunctaria	15,464	40.62	7.86	40.24	11.28	80.86	0.00	−0.18
H. luctuosalis	15,342	39.64	7.78	40.78	11.80	80.42	−0.01	−0.21
M. exigua	15,262	40.47	7.55	41.12	10.86	81.59	−0.01	−0.18
C. perspectalis	15,232	39.81	7.69	41.11	11.38	80.92	−0.02	−0.19
P. gossypiella	15,202	40.08	7.68	40.61	11.63	80.69	−0.01	−0.20
D. ustalella	15,410	39.05	7.75	42.07	11.13	81.12	−0.04	−0.18
S. sinensis	15,216	38.70	7.74	42.15	11.42	80.85	−0.04	−0.19
L. lepita	15,167	39.87	7.69	41.31	11.13	81.18	−0.02	−0.18
I. eugenia	15,206	39.45	7.43	42.05	11.07	81.50	−0.03	−0.20
P. apollonius	15,245	40.11	7.54	41.33	11.03	81.44	−0.01	−0.19
E. cinereus	15,285	39.75	7.45	41.86	10.95	81.61	−0.03	−0.19

Table 4. *Cont.*

Species	Size (bp)	A%	G%	T%	C%	A + T%	AT Skew	GC Skew
H. puera	15,350	40.59	7.73	40.61	11.08	81.20	0.00	−0.18
G. dimorpha	15,813	39.99	7.77	40.85	11.39	80.84	−0.01	−0.19
G. molesta	15,776	40.36	7.74	40.88	11.02	81.24	−0.01	−0.17
C. sasakii	15,611	42.00	7.75	39.50	10.75	81.50	0.03	−0.16
C. maeniata	15,284	39.87	7.35	42.18	10.61	82.05	−0.03	−0.18
PCG								
S. thespis	11,219	32.58	11.47	44.48	11.47	77.06	−0.15	0.00
E. trimenii	11,208	33.65	10.25	46.14	9.84	79.79	−0.16	0.02
A. assama	11,211	33.01	10.76	45.78	10.45	78.79	−0.16	0.01
P. colligata	11,172	33.92	10.67	45.67	9.75	79.59	−0.15	0.05
S. morio	11,179	34.09	10.51	45.75	9.65	79.84	−0.15	0.04
E. laeta	11,213	33.74	10.92	44.98	10.35	78.72	−0.14	0.03
S. sigillata	11,205	33.82	10.39	46.27	9.51	80.09	−0.16	0.04
A. pallidipennis	11,214	33.81	10.62	45.75	9.82	79.56	−0.15	0.04
P. prasinana	11,209	33.73	10.91	45.66	9.70	79.39	−0.15	0.06
E. adulatricoides	11,211	33.81	10.76	45.60	9.84	79.41	−0.15	0.04
E. narcissus	11,203	34.05	10.66	45.32	9.97	79.37	−0.14	0.03
P. grotei	11,197	33.65	10.66	45.40	10.29	79.05	−0.15	0.02
T. albicostata	11,202	33.86	10.77	45.15	10.21	79.01	−0.14	0.03
P. parvula	11,206	34.14	10.64	45.07	10.15	79.21	−0.14	0.02
L. zampa	11,179	34.64	10.24	45.56	9.56	80.20	−0.14	0.03
S. subpunctaria	11,223	33.96	10.97	45.10	9.97	79.06	−0.14	0.05
H. luctuosalis	11,193	33.75	10.83	45.05	10.36	78.80	−0.14	0.02
M. exigua	11,202	34.30	10.41	45.92	9.37	80.22	−0.14	0.05
C. perspectalis	11,159	34.04	10.70	45.46	9.79	79.50	−0.14	0.04
P. gossypiella	11,191	33.84	10.59	45.55	10.03	79.39	−0.15	0.03
D. ustalella	11,209	33.46	10.65	45.87	10.02	79.33	−0.16	0.03
S. sinensis	11,187	33.59	10.49	45.99	9.92	79.58	−0.16	0.03
L. lepita	11,197	34.24	10.51	45.69	9.56	79.93	−0.14	0.05
I. eugenia	11,177	34.24	10.15	10.15	9.49	44.39	0.54	0.03
P. apollonius	11,201	34.73	10.02	45.99	9.27	80.72	−0.14	0.04
E. cinereus	11,183	34.46	10.31	45.86	9.37	80.32	−0.14	0.05
H. puera	11,196	34.24	10.75	45.51	9.49	79.75	−0.14	0.06
G. dimorpha	11,239	33.57	10.93	45.13	10.37	78.70	−0.15	0.03
G. molesta	11,172	33.66	10.79	45.59	9.95	79.25	−0.15	0.04
C. sasakii	11,178	34.83	10.42	45.00	9.75	79.83	−0.13	0.03
C. maeniata	11,195	34.33	9.94	46.43	9.30	80.76	−0.15	0.03
tRNA								
S. thespis	1463	40.26	8.27	40.26	11.21	80.52	0.00	−0.15
E. trimenii	1462	41.45	10.94	40.36	6.98	81.81	0.01	0.22
A. assama	1466	41.00	11.39	39.56	8.05	80.56	0.02	0.17
P. colligata	1475	41.15	10.58	40.20	8.07	81.35	0.01	0.13
S. morio	1463	41.76	10.53	39.85	7.86	81.61	0.02	0.15
E. laeta	1468	41.14	11.31	39.44	8.11	21.27	0.03	0.16
S. sigillata	1478	42.69	10.42	39.51	7.37	82.20	0.04	0.17
A. pallidipennis	1458	42.04	10.29	40.05	7.61	82.09	0.02	0.15
P. prasinana	1465	41.64	11.19	39.25	7.92	80.89	0.03	0.17
E. adulatricoides	1476	42.21	10.50	39.63	7.66	81.84	0.03	0.16
E. narcissus	1453	41.57	10.94	39.78	7.71	81.35	0.02	0.17
P. grotei	1494	42.30	10.58	39.49	7.63	81.79	0.03	0.16
T. albicostata	1472	42.39	10.39	39.33	7.88	81.72	0.04	0.14
P. parvula	1463	41.49	10.73	39.78	8.00	81.27	0.02	0.15
L. zampa	1472	41.44	10.87	40.22	7.47	81.66	0.01	0.19
S. subpunctaria	1470	41.84	10.34	39.66	8.16	81.50	0.03	0.12
H. luctuosalis	1467	41.99	10.70	39.54	7.77	81.53	0.03	0.16
M. exigua	1474	42.13	10.58	39.82	7.46	81.95	0.03	0.17
C. perspectalis	1478	41.14	10.01	40.60	8.25	81.74	0.01	0.10
P. gossypiella	1481	41.46	10.60	40.18	7.77	81.64	0.02	0.15

Table 4. *Cont.*

Species	Size (bp)	A%	G%	T%	C%	A + T%	AT Skew	GC Skew
D. ustalella	1485	41.95	10.10	40.27	7.68	82.22	0.02	0.14
S. sinensis	1471	41.26	10.74	40.24	7.75	81.50	0.01	0.16
L. lepita	1456	41.69	11.13	39.35	7.83	81.04	0.03	0.17
I. eugenia	1468	41.49	10.49	40.05	7.97	81.54	0.02	0.14
P. apollonius	1449	42.03	10.77	39.27	7.94	81.30	0.03	0.15
E. cinereus	1474	41.99	10.65	39.76	7.60	81.75	0.03	0.17
H. puera	1472	41.64	10.80	39.47	8.08	81.11	0.03	0.14
G. dimorpha	1465	41.71	10.58	39.80	7.92	81.51	0.02	0.14
G. molesta	1469	41.80	10.82	39.28	8.10	81.08	0.03	0.14
C. sasakii	1457	42.07	10.64	39.60	7.69	81.67	0.03	0.16
C. maeniata	1475	42.24	9.83	40.47	7.46	82.71	0.02	0.14
rRNA								
S. thespis	2070	40.87	5.31	43.00	10.82	83.87	−0.03	−0.34
E. trimenii	2100	44.14	9.62	41.00	5.19	85.14	0.04	0.30
A. assama	2150	43.40	10.65	40.98	4.98	84.38	0.03	0.36
P. colligata	2090	42.34	9.90	42.92	4.83	85.26	−0.01	0.34
S. morio	2152	43.08	10.36	41.73	4.83	84.81	0.02	0.36
E. laeta	2166	41.74	10.66	42.94	4.66	84.68	−0.01	0.39
S. sigillata	2063	43.04	9.94	41.98	5.04	85.02	0.01	0.33
A. pallidipennis	2081	43.97	10.62	40.27	5.14	84.24	0.04	0.35
P. prasinana	2089	44.38	9.57	40.93	5.12	85.31	0.04	0.30
E. adulatricoides	2144	42.96	10.49	41.60	4.94	84.56	0.02	0.36
E. narcissus	2041	42.43	10.63	41.79	5.14	84.22	0.01	0.35
P. grotei	2154	42.06	11.28	41.69	4.97	83.75	0.00	0.39
T. albicostata	2073	43.13	10.37	41.24	5.26	84.37	0.02	0.33
P. parvula	2158	43.19	9.78	41.98	5.05	85.17	0.01	0.32
L. zampa	2062	42.14	10.48	42.34	5.04	84.48	0.00	0.35
S. subpunctaria	2088	42.24	10.01	42.77	4.98	85.01	−0.01	0.34
H. luctuosalis	2155	44.73	10.02	40.42	4.83	85.15	0.05	0.35
M. exigua	2139	43.71	9.54	41.84	4.91	85.55	0.02	0.32
C. perspectalis	2107	44.47	10.39	40.10	5.03	84.57	0.05	0.35
P. gossypiella	2133	44.49	10.69	39.90	4.92	84.39	0.05	0.37
D. ustalella	2236	45.75	9.03	40.56	4.65	86.31	0.06	0.32
S. sinensis	2168	43.82	10.75	40.68	4.75	84.50	0.04	0.39
L. lepita	2105	45.13	10.12	39.81	4.94	84.94	0.06	0.34
I. eugenia	2052	45.52	10.28	39.08	5.12	84.60	0.08	0.34
P. apollonius	2182	42.76	10.45	41.61	5.18	84.37	0.01	0.34
E. cinereus	2064	43.36	10.08	41.72	4.84	85.08	0.02	0.35
H. puera	2038	42.44	10.75	41.81	5.00	84.25	0.01	0.37
G. dimorpha	2181	43.83	10.09	41.13	4.95	84.96	0.03	0.34
G. molesta	2157	42.10	6.77	42.88	8.25	81.08	0.03	−0.10
C. sasakii	2143	42.56	9.43	42.70	5.32	85.26	0.00	0.28
C. maeniata	2125	43.25	9.98	41.98	4.8	85.23	0.01	0.35
A + T-rich region								
S. thespis	331	41.99	3.93	48.34	5.74	90.33	−0.07	−0.19
E. trimenii	352	43.75	2.84	46.88	6.25	90.63	−0.03	−0.38
A. assama	332	40.96	2.11	49.70	7.23	90.66	−0.10	−0.55
P. colligata	358	43.58	1.68	51.96	2.79	95.54	−0.09	−0.25
S. morio	316	44.30	2.53	48.42	4.75	92.72	−0.04	−0.30
E. laeta	372	45.16	4.30	46.24	4.30	91.40	−0.01	0.00
S. sigillata	196	48.47	2.04	44.90	4.59	93.37	0.04	−0.38
A. pallidipennis	338	43.79	1.78	50.00	4.44	93.79	−0.07	−0.43
P. prasinana	306	43.46	1.63	49.67	5.23	93.13	−0.07	−0.52
E. adulatricoides	341	46.04	2.64	46.63	4.69	92.67	−0.01	−0.28
E. narcissus	432	47.22	1.39	48.84	2.55	96.06	−0.02	−0.29
P. grotei	361	43.21	2.77	49.31	4.71	92.52	−0.07	−0.26

Table 4. *Cont.*

Species	Size (bp)	A%	G%	T%	C%	A + T%	AT Skew	GC Skew
T. albicostata	341	41.64	2.93	50.15	5.28	91.79	−0.09	−0.29
P. parvula	343	44.02	2.04	49.85	4.08	93.87	−0.06	−0.33
L. zampa	640	47.03	0.47	50.00	2.50	97.03	−0.03	−0.68
S. subpunctaria	463	43.41	1.94	52.48	2.16	95.89	−0.09	−0.05
H. luctuosalis	396	40.91	1.52	52.53	5.05	93.44	−0.12	−0.54
M. exigua	340	43.82	0.88	50.59	4.71	94.41	−0.07	−0.69
C. perspectalis	316	45.57	2.53	49.68	2.22	95.25	−0.04	0.07
P. gossypiella	309	41.10	2.59	53.72	2.59	94.82	−0.13	0.00
D. ustalella	321	42.06	2.80	52.34	2.80	94.40	−0.11	0.00
S. sinensis	271	42.80	0.74	53.51	2.95	96.31	−0.11	−0.60
L. lepita	337	43.32	0.89	53.12	2.67	96.44	−0.10	−0.50
I. eugenia	402	45.27	1.24	49.25	4.23	94.52	−0.04	−0.55
P. apollonius	345	38.84	6.96	46.09	8.12	84.93	−0.09	−0.08
E. cinereus	353	43.34	1.13	51.27	4.25	94.61	−0.08	−0.58
H. puera	439	47.15	1.14	50.11	1.59	97.26	−0.03	−0.16
G. dimorpha	848	41.63	1.30	54.83	2.24	96.46	−0.14	−0.27
G. molesta	836	46.65	1.32	49.28	2.75	95.93	−0.03	−0.35
C. sasakii	656	48.63	3.35	44.21	3.81	92.84	0.05	−0.06
C. maeniata	358	47.77	2.79	47.49	1.96	95.26	0.00	0.17

3.2. PCGs and Codon Usage

The PCGs and codon usage of *S. thespis* were analyzed (Table 3). In the mitochondrial genome of *S. thespis*, 13 PCGs were 11, 219 bp in length, accounting for 73.32% of the complete mitogenome. Nine PCGs (*nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad6* and *cob*) were encoded by the H-chain and four PCGs (*nad5*, *nad4*, *nad4L* and *nad1*) were encoded by the L-chain. *nad5* and *atp8* were the longest and the shortest genes, with 1746 bp and 165 bp, respectively. Among the 13 PCGs, the initiation codon of cytochrome c oxidase subunit 1 (*cox1*) was CGA, and the rest were ATN (ATT, ATG, ATC, ATA and ATG). *Nad2* and *nad3* started with ATT, *cox2*, *cox3*, *atp6*, *nad4*, *nad4l*, *cob*, *nad1* and *cyt b* started with ATG, *atp8* and *nad6* started with ATC and *nad5* started with ATA. Compared with the diversity of the start codons, there were only four types of stop codons in Lepidoptera mitochondrial genomes, namely TAA, TAG, TA and T, among which TAA was the most frequently used stop codon. In *S. thespis*, two genes (*cox1* and *cox2*) had an incomplete stop codon T, while the rest terminated with a canonical stop codon TAA. The use of T as a stop codon is common in Lepidoptera, especially in *cox1* and *cox2* genes, which may be formed through polyadenylation during transcription [26].

The codon usage analysis of *S. thespis* showed that Phe, Ile, Leu and Asn were the most frequent amino acids, while Trp was the least (Figure 2). The amino acid distribution of mitochondrial genomes from 30 different Lepidoptera species were compared, and it was found that the distribution of codons in these species was consistent (Figure 2). The relative synonymous codon usage (RSCU) values of 13 PCGs in *S. thespis* were calculated. The usage of UUA, UCU, CGA, ACU and GCU codons were higher, while CCG, AGG, CUG, ACG and UCG were lower. The usage frequency of AT in the third codon was significantly higher than that of CG, which is highly conserved in insect mitogenomes [27,28].

3.3. Ribosomal RNA and Transfer RNA Genes

The complete mitochondrial genome of *S. thespis* contained two rRNA (*rrnL* and *rrnS*) genes and 22 tRNA genes. The 16S rRNA gene (*rrnL*) was 1294 bp between *trnL1* and *trnV*, and the 12S rRNA gene (*rrnS*) was 776 bp between *trnV* and the A + T-rich region. The AT content of two rRNA genes accounted for 83.87%, and the AT skewness and GC skew skewness were −0.03 and −0.34, respectively (Table 4).

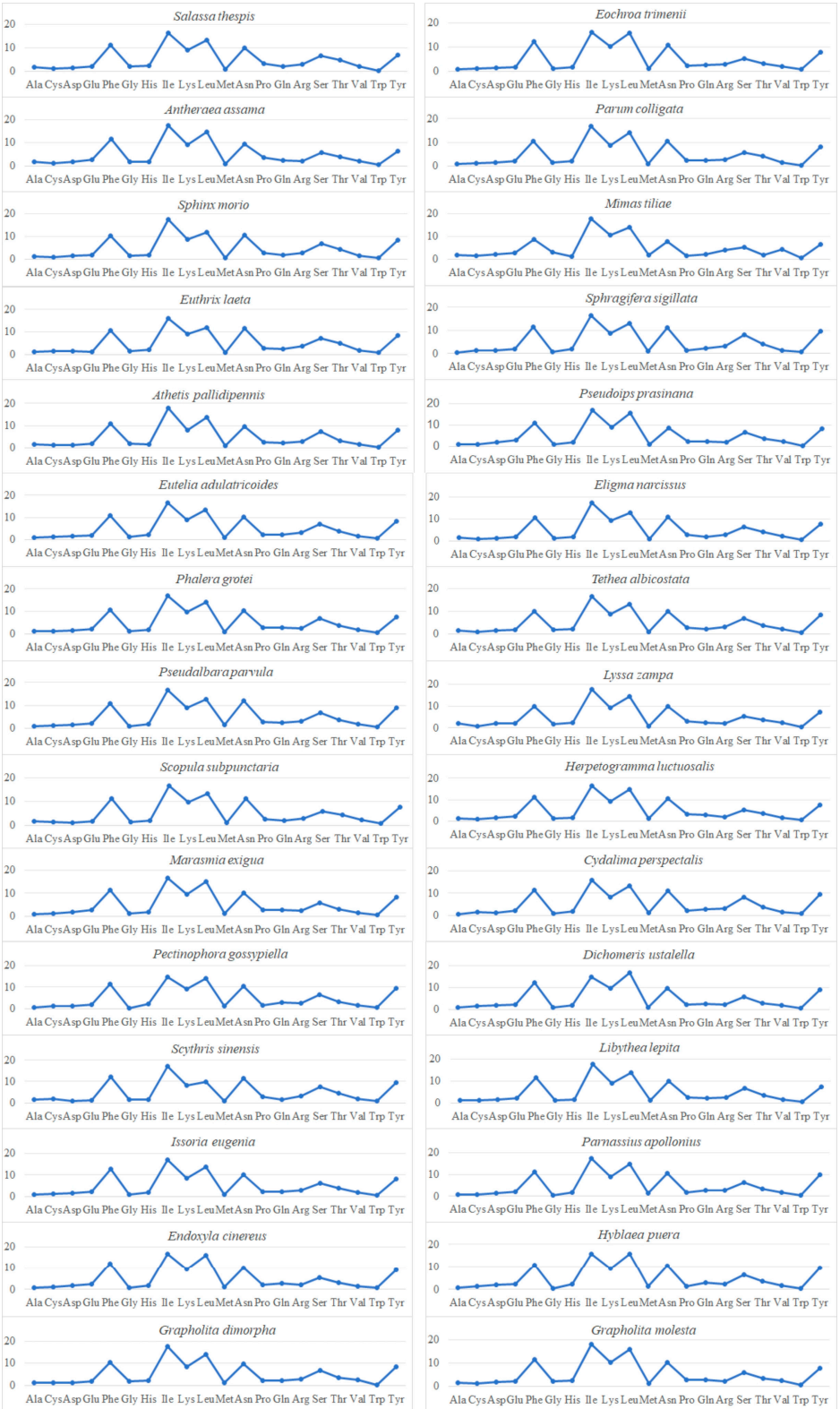

Figure 2. Codon distribution patterns in various Lepidoptera species. The y-coordinate is the proportion of codons per 100 codons.

The length of 22 tRNA genes in *S. thespis* ranged from 64 bp (*trnC*, *trnY*, *trnR*, *trnF* and *trnP*) to 71 bp (*trnK*). The secondary structures of the tRNAs were typical cloverleaf structures, except *trnS1*, which lacked a dihydrouridine arm, which is similar to the features of most other Lepidoptera tRNAs (Figure 3). A total of 23 mismatched base pairs

were identified in the *S. thespis* tRNAs, including 19 GU pairs and four UU pairs. The mismatched tRNAs were mainly the *trnM*, *trnI*, *trnQ*, *trnW*, *trnC*, *trnL1*, *trnL2*, *trnG*, *trnA*, *trnS1*, *trnS2*, *trnF*, *trnT* and *trnP* genes. The gene arrangement of tRNAs is always conserved in Lepidoptera, and the rearrangement is mainly concentrated in *trnM*, *trnI* and *trnQ* gene clusters. In *S. thespis*, it is *trnM-trnI-trnQ*, which differs from the ancestral order *trnI-trnQ-trnM* [29].

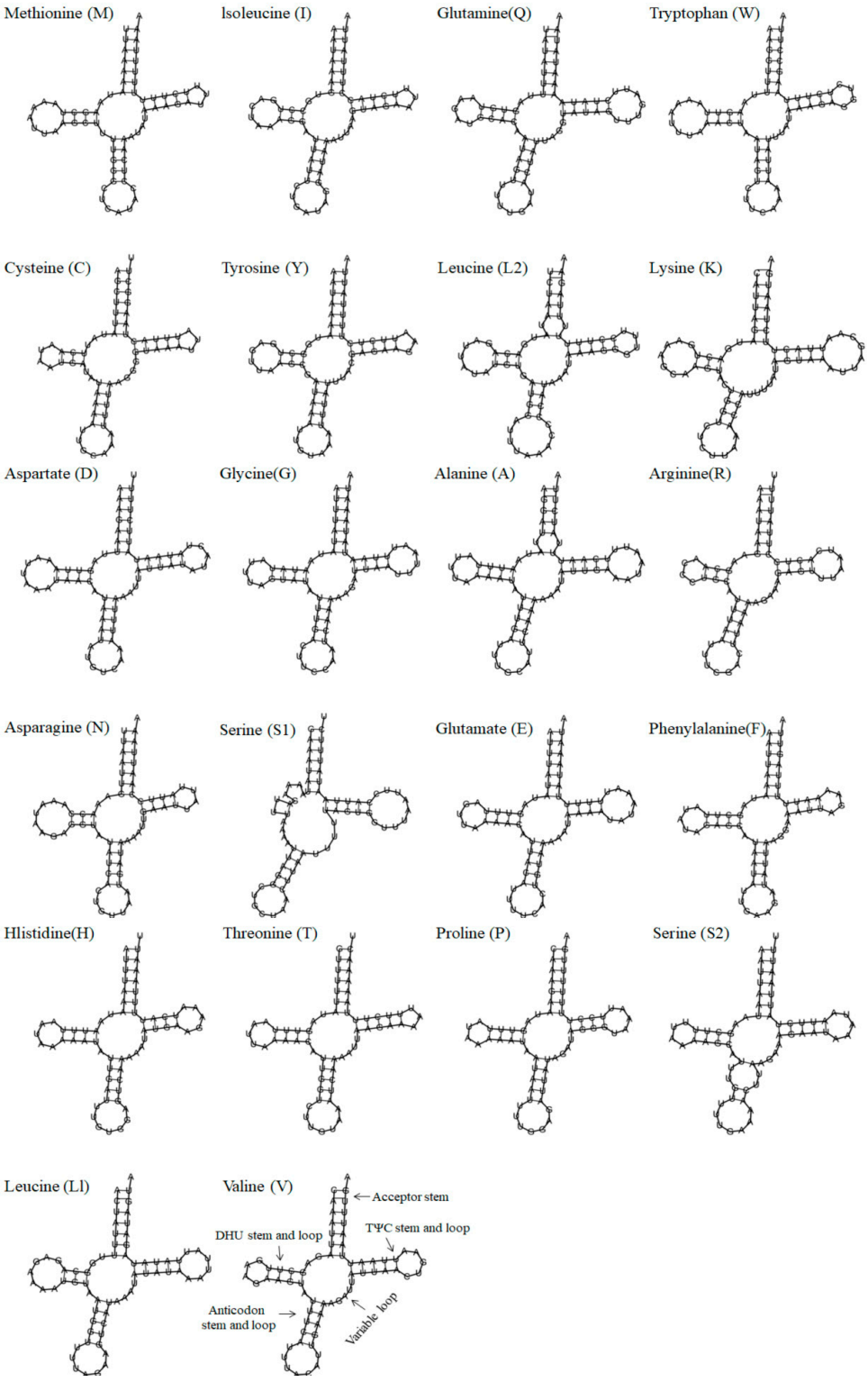

Figure 3. Putative secondary structures of the 22 tRNA genes of the *S. thespis* mitogenome [28].

3.4. Overlapping and Intergenic Spacer Regions

Ten overlapping regions with a total of 33 bp, ranging from 1 to 8 bp, were identified in the mitochondrial genome of *S. thespis* (Table 3). The longest overlapping region was between *trnW* and *trnC*, and the shortest was between *atp6* and *cox3*, *trnA* and *trnR*, *trnR* and *trnN* and *nad6* and *cob*. In general, there is a 7 bp overlapping sequence ("ATGATAG" or "ATGATAA") between the 3′ end of the *atp8* gene and the 5′ end of the *atp6* gene in the mitochondrial genomes of Lepidopteran [30]. Except for the Micropterigoidea family, which had a 7 bp overlap sequence of ATGATAG, the sequence of other Lepidoptera insects was ATGATAA, and the sequence of *S. thespis* here was ATGATAA (Figure 4). Fifteen intergenic spacer regions with a total of 222 bp, ranging from 1 to 50 bp, were identified in the mitochondrial genome of *S. thespis* (Table 3). The longest overlapping region was between *trnQ* and *nad2*, and the shortest was between *nad1* and *trnL1*. The 18 bp spacer between *trnS2* and *nad1* contained the motif ATACTAA, which is also present in many Lepidoptera mitochondrial genomes and may be a mitochondrial transcription termination peptide-binding site [31–33].

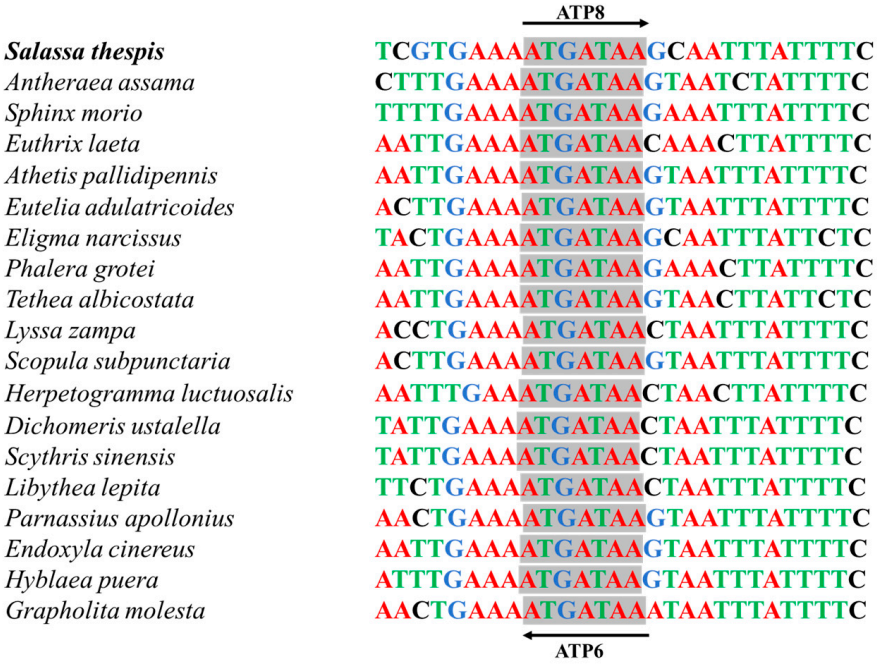

Figure 4. Alignment of overlapping region between *atp8* and *atp6* across Lepidoptera.

3.5. The A + T-Rich Region

The length of the A + T-rich region (control region, CR) of *S. thespis* mitochondria was 331 bp, starting from 14,972 bp to 15,302 bp, and was located between the *rrnS* and *trnM* genes (Table 3). Compared with other regions of the *S. thespis* mitochondrial genome, the AT content in the CR was as high as 90.33%, and the AT skewness and GC skewness in this region were −0.07 and −0.19, respectively (Table 4). Like most Lepidopterans [34,35], some conserved motifs were found in the CR of the *S. thespis* mitochondrial genome, including a 17 bp poly-T guided by "ATAGA", the AT-rich area and a poly-A element. The "ATAGA" sequence was located at the beginning of the CR, near the 5′ end of *12S rRNA* (Figure 5). The "ATAGA" motif is usually considered to be the precise location of the origin of replication, and the poly-T sequence is considered to be the structural signal for protein recognition in the initiation of replication [36].

rrnS-14,971 bp-ATTCATGTATTTTTTCACATAGATTTTTTTTTTTTTTTTTATATAATAAA
ATATTTAATAGAAATTAATAAATTTTGAATATTTTCTCTTATTTTTTCTTCATAATATTCA
ATGTAAATATAAAATGGCTATTTGAATTTTTATAATTATCCAAATTATATTATATTAAAAT
AATTAATATTAATTTTTTTAGTAATTTATTATATTAATATATATATATATACATGTAAATATT
TAATATAATAAAATTAAAAATTTGATAATATTTTACTCAAAAATATAATCTTAAACCGTT
TTTCTTAATTTTTCATATAAATAAAAAAAAAATAAGA-15,302 bp-*trnM*

Figure 5. Features present in the A + T-rich region of *S. thespis*. The ATAGA motif is marked red. The poly-T is marked with a dotted line. The microsatellite TA repeats is indicated by wavy lines and the poly-A is underlined.

3.6. Phylogenetic Analyses

A phylogenetic tree was constructed based on 13 PCGs sequences of mitogenomes from 31 species of Lepidoptera (containing 12 superfamilies, which were Bombycoidea, Noctuoidea, Geometroidea, Drepanoidea, Pyraloidea, Hyblaeoidea, Gelechioidea, Hesperioidea, Papilionoidea, Cossoidea, Copromorphoidea and Tortricoidea) using BI and ML methods (Figures 6 and 7). The results obtained from the two methods were consistent, indicating that *Samia canningi* and *Rhodinia fugax* were sister groups. *S. thespis* was closely related to *Samia canningi* and *Rhodinia fugax*, all of which belonged to Saturniidae, Bombycoidea. Saturniidae, Sphingidae, and Lasiocampidae belonged to Bombycoidea, and Bombycoidea was the closest to Noctuoidea. Additionally, three species within the Saturniidae showed the following relationship: (*S. thespis* + (*Rhodinia fugax* + *Samia canningi*)).

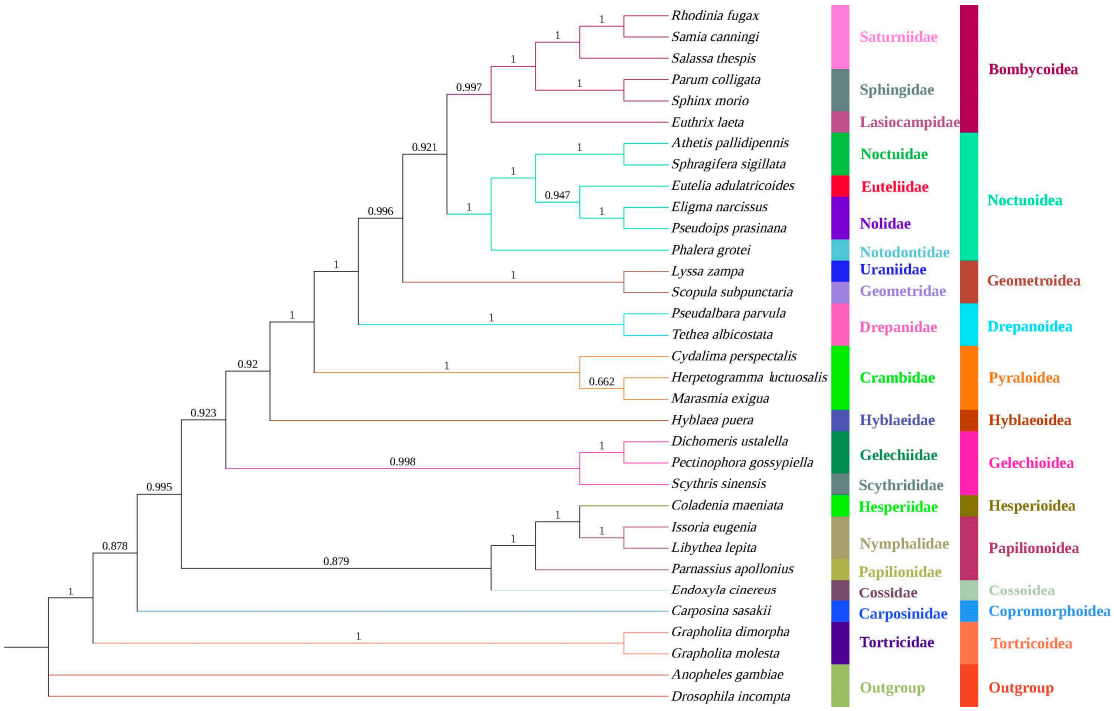

Figure 6. BI phylogenetic trees of Lepidoptera species based on the protein-coding genes. Numbers above each node indicate Bayesian posterior probability values [5].

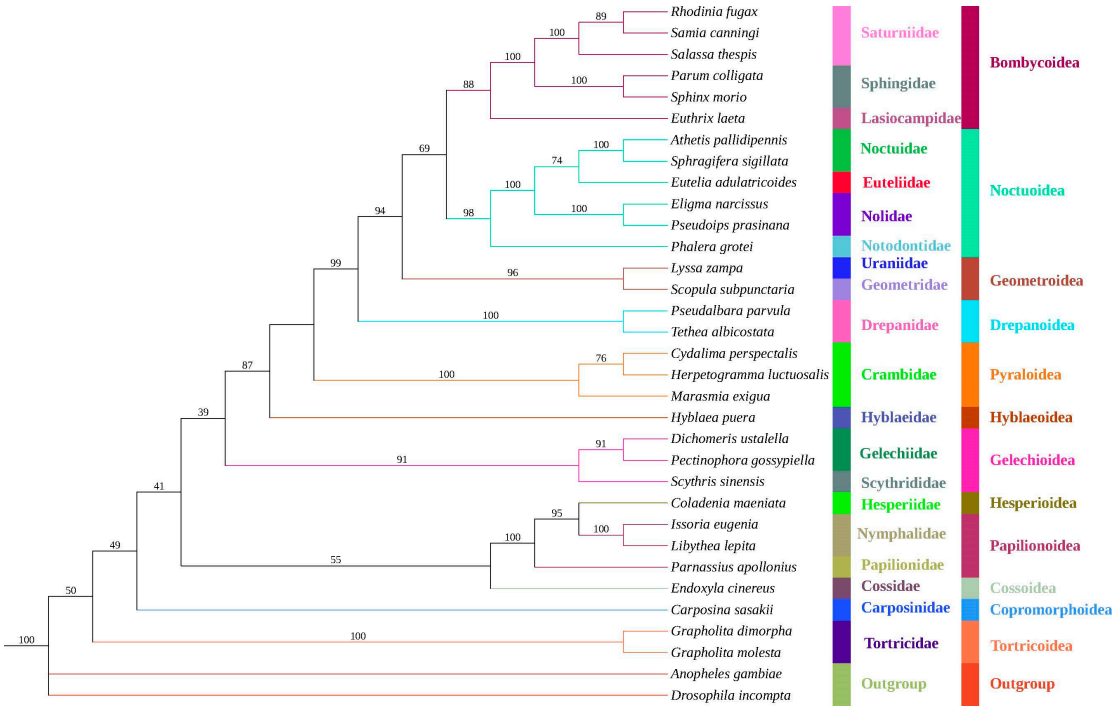

Figure 7. ML phylogenetic trees of Lepidoptera species based on the protein-coding genes. Numbers above each node indicate ML bootstrap values [5].

4. Discussion

The mitochondrial genome contains a wealth of genetic information, including gene length, gene order, base bias, genetic codon composition, control region (CR) and repeat region, etc., which play an important role in better understanding the evolutionary characteristics and diversity of insects. Mitochondrial genomes of Lepidoptera insects are generally 15–16 kb in size and usually contain 37 genes and a non-coding CR. Here, the mitochondrial genome of *S. thespis* was 15,302 bp in length, containing 37 genes, including 13 PCGs, 2 rRNAs, 22 tRNAs and a CR.

The AT content in the mitochondrial genome of Lepidoptera generally ranges from 76% to 86%, with a high of 86.6% in Micropterygidae. In *S. thespis*, the AT content was 78.8%, indicating that the mitochondrial genome of *S. thespis* had obvious AT bias. The AT skewness and GC skewness of the whole genome of *S. thespis* were both negative, which indicate the occurrence of more Ts than As and more Cs than Gs. In 13 PCGs of *S. thespis*, the AT content of the third codon was significantly higher than that of GC.

The length of tRNA in insect mitochondrial genome is generally 60–73 bp, and the length of *trnK* is stable, mostly 71 bp. In *S. thespis*, the length of *trnK* is also 71 bp. Among the 22 tRNAs in insect mitochondria, except for *Adoxophyes honmai*, most *trnS1* genes are unable to form stable cloverleaf structures [37]. In *S. thespis*, the secondary structures of the other 21 tRNAs were all typical cloverleaf structures, except that the dihydrouridine arm of *trnS1* was deleted.

The A + T-rich region is named for having the highest AT content in the mitochondrial genome. This region is usually considered to be the main regulatory region of mitochondrial genome replication and transcription, so it is also called the CR. In addition, the D-loop region is frequently used in some studies because of its three-strand loop structure during replication. The CR of *S. thespis* (13972–15302 bp) had three conserved structures: ATAGA motif, poly-T and AT microsatellite sequence. ATAGA was located at the beginning of the CR, near the 5′ end of *12S rRNA*. The ATAGA motif of some Lepidoptera groups was mutated in individual bases, and structures such as ATAG, ATAGAA, ATGGA, ACAGA and ATATAA appeared. The poly-T followed the ATAGA motif; however, there were some

base mutations of the poly-T structure in Adeloidea, Micropterigoidea and Tischerioidea, such as TTATATATATTTAA.

There is always a 7 bp overlapping region between the 3′ end of *atp8* and the 5′ end of *atp6* in insect mitochondrial genomes. In Diptera, Grylloblattodea, Megaloptera and Raphidioptera, the overlapping region is ATGATAA [38–40]. In Lepidoptera, except for Micropterigoidea, where this overlapping region is ATGATAG, the other species are ATGATAA, and the *S. thespis* was also ATGATAA. However, in Hymenoptera, there are even five types in this region [41,42].

The phenomenon of mitochondrial gene rearrangement is often one of the elements in the analysis of phylogenetic relationships between species [2,43]. Two types of gene rearrangements are recognized to mainly occur in Lepidoptera mitochondrial genomes. The gene cluster *trnM-trnI-trnQ* located at the 5′ end is the main rearrangement region, which is consistent with the first sequenced Lepidoptera insect, *Bombyx mori*. The other rearrangement order *trnI-trnQ-trnM* occurs in primitive Lepidoptera, such as Nepticuloidea, Hepialoidea and Adeloidea, which is consistent with Drosophila [44–46]. In *S. thespis*, it is in the same *trnM-trnI-trnQ* order as the *Bombyx mori*.

Molecular phylogenetic analysis based on mitochondrial genomes provides more reference information for taxonomy. There is currently no literature on the molecular level classification of *S. thespis*. Moreover, 31 species of Lepidoptera containing 12 superfamilies were used to construct a phylogenetic tree based on 13 PCGs sequences of mitogenomes using BI and ML methods. *S. thespis* was closely related to *Samia canningi* and *Rhodinia fugax*, all of which belonged to Saturniidae, Bombycoidea. This is consistent with the morphological and NCBI lineage of *S. thespis*. Of course, data on Lepidoptera mitochondria are limited, and further taxonomic sampling of Lepidoptera species is needed to better understand the phylogenetic relationships between different taxa.

5. Conclusions

The mitochondrial genome of *S. thespis* was determined and characterized, including the genome structure, base composition, PCGs, codon usage, RNA genes, overlapping and intergenic spacer regions, CR region and phylogenetic analysis. The comprehensive analysis of *S. thespis* mitochondrial genome provides the data for the evolution and diversity research of Lepidoptera species. However, the Lepidoptera population is large, and more molecular or mitochondrial data are still needed to further explore its phylogeny and classification.

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References

1. Wolstenholme, D.R. Animal mitochondrial DNA: Structure and evolution. *Int. Rev. Cytol.* **1992**, *141*, 173–216. [CrossRef] [PubMed]
2. Cameron, S.L. Insect mitochondrial genomics: Implications for evolution and phylogeny. *Annu. Rev. Entomol.* **2014**, *59*, 95–117. [CrossRef] [PubMed]
3. Song, S.N.; Tang, P.; Wei, S.J.; Chen, X.X. Comparative and phylogenetic analysis of the mitochondrial genomes in basal hymenopterans. *Sci. Rep.* **2016**, *6*, 20972. [CrossRef] [PubMed]
4. Wu, J.; Xu, D.; Wei, X.; Liao, W.; Li, X.; Zhuo, Z. Characterization of the complete mitochondrial genome of the longhorn beetle, *Batocera horsfieldi* (Coleoptera, Cerambycidae) and its phylogenetic analysis with suitable longhorn beetles. *Zookeys* **2023**, *1168*, 387–402. [CrossRef] [PubMed]
5. Liu, D.; Zhang, H.; Fu, S.; Wang, Y.; Zhao, W.; Zhao, Q. The complete mitochondrial genomes of *Aelia sibirica* and *A. fieberi* (Hemiptera, Pentatomidae), and phylogenetic implications. *Diversity* **2024**, *16*, 111. [CrossRef]
6. Chen, D.B.; Zhang, R.S.; Bian, H.X.; Li, Q.; Xia, R.X.; Li, Y.P.; Liu, Y.Q.; Lu, C. Comparative mitochondrial genomes provide new insights into the true wild progenitor and origin of domestic silkworm *Bombyx mori*. *Int. J. Biol. Macromol.* **2019**, *131*, 176–183. [CrossRef]
7. Kawahara, A.Y.; Breinholt, J.W. Phylogenomics provides strong evidence for relationships of butterflies and moths. *Proc. Biol. Sci.* **2014**, *281*, 20140970. [CrossRef]
8. Yang, X.; Cameron, S.L.; Lees, D.C.; Xue, D.; Han, H. A mitochondrial genome phylogeny of owlet moths (Lepidoptera: Noctuoidea), and examination of the utility of mitochondrial genomes for lepidopteran phylogenetics. *Mol. Phylogenet. Evol.* **2015**, *85*, 230–237. [CrossRef] [PubMed]
9. Park, J.S.; Kim, M.J.; Jeong, S.Y.; Kim, S.S.; Kim, I. Complete mitochondrial genomes of two gelechioids, *Mesophleps albilinella* and *Dichomeris ustalella* (Lepidoptera: Gelechiidae), with a description of gene rearrangement in Lepidoptera. *Curr. Genet.* **2016**, *62*, 809–826. [CrossRef]
10. Thurman, J.H. Beyond the pest: Life history, ecology and ethnoentomology of the giant wood moth (*Endoxyla cinereus*). *Austral. Ecol.* **2022**, *47*, 733–747. [CrossRef]
11. Huang, W.; Zhang, C.; Zhang, T.; Xu, Y.; Xu, S.; Tian, L.; Li, H.; Cai, W.; Song, F. Features and evolution of control regions in leafroller moths (Lepidoptera: Tortricidae) inferred from mitochondrial genomes and phylogeny. *Int. J. Biol. Macromol.* **2023**, *236*, 123928. [CrossRef] [PubMed]
12. Zhu, L.; Han, Y.; Hou, Y.; Huang, Z.; Wang, M.; Chiba, H.; Chen, L.; Fan, X. Mitogenomic phylogenetic analyses provide novel insights into the taxonomic problems of several hesperiid taxa (Lepidoptera: Hesperiidae). *Sci. Rep.* **2023**, *13*, 7901. [CrossRef] [PubMed]
13. Zheng, X.; Xu, Z.; Wang, D.; Zhou, C. Life history and mitochondrial genomes of Salassinae and Agliinae (Insecta, Lepidoptera): New insights into the loss of cocooning behaviour and phylogeny of Saturniidae. *Bull. Entomol. Res.* **2024**, *114*, 107–123. [CrossRef] [PubMed]
14. Bernt, M.; Donath, A.; Jühling, F.; Externbrink, F.; Florentz, C.; Fritzsch, G.; Pütz, J.; Middendorf, M.; Stadler, P.F. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.* **2013**, *69*, 313–319. [CrossRef] [PubMed]
15. Grant, J.R.; Enns, E.; Marinier, E.; Mandal, A.; Herman, E.K.; Chen, C.Y.; Graham, M.; Van Domselaar, G.; Stothard, P. Proksee: In-depth characterization and visualization of bacterial genomes. *Nucleic Acids Res.* **2023**, *51*, W484–W492. [CrossRef] [PubMed]
16. Lowe, T.M.; Eddy, S.R. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **1997**, *25*, 955–964. [CrossRef] [PubMed]
17. Castresana, J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* **2000**, *17*, 540–552. [CrossRef] [PubMed]
18. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
19. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef]
20. Junqueira, A.C.; Lessinger, A.C.; Torres, T.T.; da Silva, F.R.; Vettore, A.L.; Arruda, P.; Azeredo Espin, A.M. The mitochondrial genome of the blowfly *Chrysomya chloropyga* (Diptera: Calliphoridae). *Gene* **2004**, *339*, 7–15. [CrossRef]
21. Thompson, J.D.; Gibson, T.J.; Plewniak, F.; Jeanmougin, F.; Higgins, D.G. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **1997**, *25*, 4876–4882. [CrossRef] [PubMed]
22. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef] [PubMed]
23. Lanfear, R.; Frandsen, P.B.; Wright, A.M.; Senfeld, T.; Calcott, B. Partitionfinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* **2017**, *34*, 772–773. [CrossRef] [PubMed]
24. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef] [PubMed]
25. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.; Haeseler, A.V.; Jermiin, L.S. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nat. Methods* **2017**, *14*, 587–589. [CrossRef]

26. Lu, H.F.; Su, T.J.; Luo, A.R.; Zhu, C.D.; Wu, C.S. Characterization of the complete mitochondrion genome of diurnal Moth *Amata emma* (Butler) (Lepidoptera: Erebidae) and its phylogenetic implications. *PLoS ONE* **2013**, *8*, e72410. [CrossRef] [PubMed]
27. Tzeng, C.S.; Hui, C.F.; Shen, S.C.; Huang, P.C. The complete nucleotide sequence of the *Crossostoma lacustre* mitochondrial genome: Conservation and variations among vertebrates. *Nucleic Acids Res.* **1992**, *20*, 4853–4858. [CrossRef] [PubMed]
28. Wu, L.; Jiang, X.; Xie, F.; Kausar, S.; Liang, D.; Wei, G.; Zhu, B.; Wang, L.; Liu, C.; Qian, C. The mitochondrial genome of *Smerinthus planus* (Lepidoptera: Sphingidae) and its comparative analysis with other Lepidoptera species. *Zootaxa* **2020**, *4821*, 4821.3.6. [CrossRef]
29. Boore, J.L. Animal mitochondrial genomes. *Nucleic Acids Res.* **1999**, *27*, 1767–1780. [CrossRef]
30. Chen, L.; Wahlberg, N.; Liao, C.Q.; Wang, C.B.; Ma, F.Z.; Huang, G.H. Fourteen complete mitochondrial genomes of butterflies from the genus Lethe (Lepidoptera, Nymphalidae, Satyrinae) with mitogenome-based phylogenetic analysis. *Genomics* **2020**, *112*, 4435–4441. [CrossRef]
31. Taanman, J.W. The mitochondrial genome: Structure, transcription, translation and replication. *Biochim. Biophys. Acta* **1999**, *1410*, 103–123. [CrossRef]
32. Xing, L.X.; Li, P.F.; Wu, J.; Wang, K.; You, P. The complete mitochondrial genome of the endangered butterfly *Luehdorfia taibai* Chou (Lepidoptera: Papilionidae). *Mitochondrial DNA* **2014**, *25*, 122–123. [CrossRef] [PubMed]
33. Sun, Y.U.; Zhang, J.; Li, Q.; Liang, D.; Abbas, M.N.; Qian, C.; Wang, L.; Wei, G.; Zhu, B.J.; Liu, C.L. Mitochondrial genome of *Abraxas suspecta* (Lepidoptera: Geometridae) and comparative analysis with other Lepidopterans. *Zootaxa* **2017**, *4254*, 501–519. [CrossRef] [PubMed]
34. Cameron, S.L.; Whiting, M.F. The complete mitochondrial genome of the tobacco hornworm, *Manduca sexta*, (Insecta: Lepidoptera: Sphingidae), and an examination of mitochondrial gene variability within butterflies and moths. *Gene* **2008**, *408*, 112–123. [CrossRef]
35. Chen, D.B.; Zhang, R.S.; Jin, X.D.; Yang, J.; Li, P.; Liu, Y.Q. First complete mitochondrial genome of *Rhodinia species* (Lepidoptera: Saturniidae): Genome description and phylogenetic implication. *Bull. Entomol. Res.* **2022**, *112*, 243–252. [CrossRef]
36. Saito, S.; Tamura, K.; Aotsuka, T. Replication origin of mitochondrial DNA in insects. *Genetics* **2005**, *171*, 1695–1705. [CrossRef] [PubMed]
37. Lee, E.S.; Shin, K.S.; Kim, M.S.; Park, H.; Cho, S.; Kim, C.B. The mitochondrial genome of the smaller tea tortrix *Adoxophyes honmai* (Lepidoptera: Tortricidae). *Gene* **2006**, *373*, 52–57. [CrossRef]
38. Cameron, S.L.; Sullivan, J.; Song, H.; Miller, K.B.; Whiting, M.F. A mitochondrial genome phylogeny of the Neuropterida (lace-wings, alderflies and snakeflies) and their relationship to the other holometabolous insect orders. *Zool. Scr.* **2009**, *38*, 575–590. [CrossRef]
39. Foster, P.G.; de Oliveira, T.M.P.; Bergo, E.S.; Conn, J.E.; Sant'Ana, D.C.; Nagaki, S.S.; Nihei, S.; Lamas, C.E.; Gonzalez, C.; Moreira, C.C.; et al. Phylogeny of Anophelinae using mitochondrial protein coding genes. *R. Soc. Open Sci.* **2017**, *4*, 170758. [CrossRef]
40. Yang, F.; Jiang, Y.; Yang, D.; Liu, X. Mitochondrial genomes of two Australian fishflies with an evolutionary timescale of Chauliodinae. *Sci. Rep.* **2017**, *7*, 4481. [CrossRef]
41. Wei, S.J.; Shi, M.; Sharkey, M.J.; van Achterberg, C.; Chen, X.X. Comparative mitogenomics of Braconidae (Insecta: Hymenoptera) and the phylogenetic utility of mitochondrial genomes with special reference to Holometabolous insects. *BMC Genom.* **2010**, *11*, 371. [CrossRef] [PubMed]
42. Haddad, N.J. Mitochondrial genome of the Levant Region honeybee, *Apis mellifera syriaca* (Hymenoptera: Apidae). *Mitochondrial DNA Part A* **2016**, *27*, 4067–4068. [CrossRef] [PubMed]
43. Cameron, S.L.; Barker, S.C.; Whiting, M.F. Mitochondrial genomics and the new insect order Mantophasmatodea. *Mol. Phylogenet. Evol.* **2006**, *38*, 274–279. [CrossRef] [PubMed]
44. Cao, Y.Q.; Ma, C.; Chen, J.Y.; Yang, D.R. The complete mitochondrial genomes of two ghost moths, *Thitarodes renzhiensis* and *Thitarodes yunnanensis*: The ancestral gene arrangement in Lepidoptera. *BMC Genom.* **2012**, *13*, 276. [CrossRef]
45. Timmermans, M.J.; Lees, D.C.; Simonsen, T.J. Towards a mitogenomic phylogeny of Lepidoptera. *Mol. Phylogenet. Evol.* **2014**, *79*, 169–178. [CrossRef]
46. Wei, S.J.; Niu, F.F.; Du, B.Z. Rearrangement of trnQ-trnM in the mitochondrial genome of *Allantus luctifer* (Smith) (Hymenoptera: Tenthredinidae). *Mitochondrial DNA Part A* **2016**, *27*, 856–858. [CrossRef]