Journal of Insect Science (*2016*) 16(1): 9; 1–14
doi: 10.1093/jisesa/iev157
Research article

OXFORD

The Complete Mitochondrial Genome of *Brachmia macroscopa* (Lepidoptera: Gelechiidae) and Its Related Phylogenetic Analysis

Li Ma,[1] Wan-Wei Dong,[2] Guo-Fang Jiang,[2] and Xing Wang[1,3]

[1]Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Hunan Agricultural University, Changsha, Hunan 410128, China (lily.m1106@gmail.com), [2]Jiangsu Key Laboratory for Bioresource Technology, College of Life Sciences, Nanjing Normal University, Nanjing, Jiangsu, China, and [3]Corresponding author, e-mail: wangxing@hunau.edu.cn

Subject Editor: Dr. Qili Feng

Received 24 October 2015; Accepted 11 December 2015

Abstract

The sweet potato leaf folder, *Brachmia macroscopa*, is an important pest in China. The complete mitogenome, which consists of 13 protein-coding genes (PCGs), 22 transfer RNA genes, two ribosomal RNA genes, and an A + T-rich region, was sequenced and found to be 15,394 bp in length (GeneBank no. KT354968). The gene order and orientation of the *B. macroscopa* mitogenome were similar to those of other sequenced lepidopteran species. All of the PCGs started with ATN as the canonical start codon except for *cox1*, which started with CGA. In regard to stop codons, most PCGs stopped at TAA except for *cox2*, which stopped at TA, and *nad4*, which stopped at a single T. Thirteen PCGs of the available species (33 taxa) were used to demonstrate phylogenetic relationships. The ditrysian cluster was supported as a monophyletic clade at high levels by using maximum likelihood and Bayesian methods. The apoditrysian group, covering the Gelechioidea, formed a monophyletic clade with a bootstrap value of 88% and a posterior probability of 1.00. The superfamily Gelechioidea was supported as a monophyletic lineage by a posterior probability of 1.00.

Key words: *Brachmia macroscopa*; Gelechioidea; mitogenome; phylogeny

As a well-known leaf-eating pest, *Brachmia macroscopa* Meyrick, belonging to the family Gelechiidae in the superfamily Gelechioidea, occurs in many districts of China, including Shanghai, Zhejiang, Hunan, and Hainan (Wen and Wang 2010), and damages crops by feeding on large amounts of mesophyll (Wang and Tan 2011). This pest is widely distributed outside of China, in India, the Philippines, Burma, Vietnam, Korea, and Japan (Wang and Tan 2011). This pest has already led to severe crop failures and has brought great economic losses for farmers (Huang and Li 2013). In severe cases, the rate of crop damage has reached 60–85% in some fields (Wen and Wang 2010). However, its genetic characteristics have rarely been reported. To improve the management of *B. macroscopa*, it is important to know more information about this pest, including its genetic characteristics and phylogenetic position.

Information can be inferred from the mitochondrial genome, both for phylogenetic analysis and evolutionary biology, and it can help us to understand an insects' phylogenetic position. Compared with individual genes, the complete mitochondrial genome can be more informative, and can also provide more information on genome level characteristics; for instance, gene arrangement, gene content, base composition genetic codon variation, and transfer RNA (tRNA) and ribosomal RNA (rRNA) gene secondary structures. Lepidopteran mitochondrial DNA (mtDNA) is typically a circular molecule encoding a set of 13 protein-coding genes (PCGs), 22 tRNAgenes, and two rRNA genes (Wolstenholme 1992, Boore 1999). A major non-coding element, called the control (A + T-rich) region, is always present (Lewis et al. 1995, Zhang et al. 1995, Inohira et al. 1997, Shao et al. 2001), and this is the site of gene replication and the initiation of genome transcription (Boore 1999, Taanman 1999). The mtDNA datasets have become the most helpful markers in phylogenetics, phylogeography, and genetic population studies (Avise 2000, Cameron et al. 2007).

Gelechioidea, consisting of 1,425 genera and 16,250 described species worldwide, is one of the largest and the most important lepidopteran taxa (Hodges 1998). It includes some important insect pests and occupies a very important position in the evolutionary tree of Lepidoptera. The mitogenomes of Gelechioidea have rarely been studied, with only a few sequenced mitogenomes

© The Author 2016. Published by Oxford University Press on behalf of the Entomological Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

available from five gelechioid species (09-2015), including four that are incomplete: *Oegoconia novimundi* (KJ508036), *Perimede* sp. (KJ508041), *Ethmia eupostica* (KJ508047), and *Endrosis sarcitrella* (KJ508037) (Timmermans et al. 2014), and a recently reported completely sequenced species, *Promalactis suzukiella* (Park et al. 2014). It seems that research on the phylogeny of Gelechioidea had always had some divergence or some unresolved points (Passoa 1995; Kaila 2004, Kaila et al. 2011; Bucheli and Wenzel 2005, Regier et al. 2009, Mutanen et al. 2010).

In this article, the complete mitochondrial genome of *B. macroscopa* was first sequenced and described in comparison with other lepidopteran insects, and the phylogenetic position of Gelechioidea was analyzed based on the mitogenomic data for the purpose of understanding whether such a complete dataset can determine deeper phylogeny among the contested lepidopterans, as well as the Gelechioidea.

Materials and Methods

Sampling and DNA Extraction

Larvae of *B. macroscopa* were collected from the field (an experimental field belonging to our laboratory and involving no endangered/protected species) around Hunan Agriculture University (Changsha, Hunan, China; 28°11′N, 113°4′E) and were bred in the laboratory with fresh water spinach (*Ipomoea aquatica* Forsk) leaves. Pupae of the second generation were collected and extracted to obtain total genomic DNA using the Wizard Genomic DNA Purification Kit (Promega, Beijing, China) according the manufacturer's instructions.

PCR Amplification and Sequencing

Specific primers were designed using Primer Premier 5 to compare with the known sequences and fragments of lepidopteran insects (Table 1). Universal primers were used for PCR-amplified short fragments of the mitogenome of *B. macroscopa* (Simon et al. 1994, Lee et al. 2006, Simon et al. 2006).

PCR amplification was carried out in 25 μL reactions that contained 0.2 μL rTaq (TaKaRa Co., Dalian, China), 1 μL DNA, 2.5 μL 10× rTaq buffer (Mg2 + free), 2.5 μL 25 mM $MgCl_2$, 2.0 μL dNTPs, and 0.5 μL of each primer. PCR conditions were 94°C for 5 min, 35 cycles at 94°C for 30 s, 50–59°C for 30 s, 72°C for 1–2.5 min, and a subsequent 10 min final extension at 72°C. The amplified fragments were sequenced directly by the appropriate primers using a commercial kit.

Bioinformatic Analysis

Sequences were proof-read and assembled using the program Geneious version 4.8.4 (Drummond et al. 2010). PCG boundaries were identified with the ORF finder (http://www.ncbi.nlm.nih.gov/orf/gorf.html). After completely sequencing the mt genome, it was annotated both by hand and by automated methods. For the written annotation, the method presented by Cameron (2014) was followed. The automated annotation was accomplished with MITOS (Bernt et al. 2013). Confirmation of the tRNA genes was verified using the tRNAscan-SE program (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Eddy 1997). Unidentified tRNAs were compared with sequences from other species. The two genes encoding the large and small rRNA subunits (*rrnL* and *rrnS*) were confirmed based on the rRNA alignments with other species published on NCBI, and the secondary structures were determined using Mfold Web Server (http://mfold.rna.albany.edu/?q = mfold).

Table 1. Primers used for amplification of the mitogenome of *B. macroscopa*

Fragment	Region	Primer (J/N)	Primer sequence (J/F) 5′→3′
F1	*rrns-trnQ*	SR-J-14610[c]	ATAATAGGGTATCTAATCCTAGT
		Gln-Re[b]	GCACAATAATTT TTGATATTAGATATAGTTTA
F2	*nad2*	J-60 [d]	GGTATTTGATCAGGAATAGTAGGAA
		N-735 [d]	CCAATAAATGGGGGTAATCCTCCTA
F3	*nad2-cox1*	J-489 [d]	GGGGGATTAAATCAAACCTC
		N-C1Re[b]	GCTGTTACAATAGTATTATAA ATTTGATCATC
F4	*cox1-cox2*	C1-J-2167[a]	TTGATTTTTCGGACATCCTGAAGT
		C1-N-3649[a]	CCGCAAATTTCTGAACATTGACCA
F5	*cox2-nad3*	C2-J[d]	CCGCAAATTTCTGAACATTGACCA
		N-5731[c]	TTTGGATCAAACCCACATTC
F6	*cox3-nad5*	C3-J-5470[c]	GCTGCAGCTTGATATTGACA
		N5-N-7793[d]	AATCCTAATCCATCTCAACCT
F7	*nad5-nad4*	N5-J-7572[c]	AAAAGGAATTTGAGCTCTTTTAGT
		N4-N-9153[c]	TGAGGTTATCAACCAGAGCG
F8	*nad4-cytb*	N4-J-8941[c]	GAAACTGGGGCTTCAACATGAGC
		N-11328[d]	GGCAAATAGGAAATATCATTC
F9	*cytb-nad1*	CB-J [d]	CATATTCAACCCGAATGATA
		N1-N-12588 [d]	AATCGAACTCCTTTTGATTTTGC
F10	*nad1-rrnL*	J-11876[c]	CGAGGTAAAGTACCACGAACTCA
		16S-N[d]	ATATGTACATATTGCCCGTC
F11	*rrnL-rrnS*	J-13900[d]	CTTGTGTATCAGAGTTTATTA
		N-12SR[d]	GTAAAAGTTCAAATAGCAAG

[a]Primers modified from Simon et al. (1994) prior to this mitogenome.
[b]Primers modified from Lee et al. (2006) prior to this mitogenome.
[c]Primers from Simon et al. (2006) prior to this mitogenome.
[d]Primers newly designed for this genome.

The comparative analysis and spread correction were performed by the software Mega 6.0 (Tamura et al. 2013) to obtain the complete mitogenome of *B. macroscopa* (Tamura et al. 2013). The related lepidopteran sibling species were analyzed by blast searches on the NCBI database. The skews of the compositions were determined using the formulas: AT skew = [A − T]/[A + T]; GC skew = [G − C]/[G + C] (Junqueira et al. 2004).

Phylogenetic Analysis

Along with the *B. macroscopa* mitochondrial genome, 29 available lepidopteran species (Son and Kim 2011, Gong et al. 2012) and four hepialoid mitogenomes (*Napialus hunanensis*, *Thitarodes pui*, *Ahamus yunnanensis*, and *Thitarodes renzhiensis*) (Cao et al. 2012, Yi et al. 2016a, 2016b) were used in the phylogenetic analysis (Table 2). The nucleic acid regions and amino acid sequences from all 13 PCGs were aligned using Mega 6.0 (Tamura et al. 2013). Gblock 0.91b with default settings was used with conserved regions of the putative amino acids (Castresana, 2000).

The 13 individual best fitting models for the nucleic acid dataset were defined with the Akaike Information Criterion (AIC) and the jModelTest 2.1.5 (Ronquist and Huelsenbeck 2003). Then, Bayesian inference (BI) analysis was performed via MrBayes v3.2.3 (Darriba et al. 2012), with the MCMC analysis run for 1,000,000 generations and a burn-in series of 1,000. In addition, the maximum likelihood (ML) method was conducted with RAxML v 8.0.2 (Stamatakis 2014), in which the 13 best fit substitution models for the 13 separate PCGs (aa sequence) were determined on the Protest web server in terms of AIC (Abascal et al. 2005).

Results

Genome Organization and Base Composition

The complete mtDNA of *B. macroscopa* was featured as a 15,394 bp closed circle (Fig. 1) and encoded 37 genes as well as containing a putative A + T-rich region. The 37 genes were organized by 13 PCGs (*cox1-3*, *nd1-6*, *atp6*, *atp8*, *nad4L*, and *cytb*), 22 tRNA genes and 2 rRNA genes (Table 3). Twenty of the genes were transcribed on the major strand (J-strand), and the other 17 were transcribed on the minor strand (N-strand).

The intergenic spacer sequence was 184 bp long in total and was made up of 16 regions, which varied from 1 to 83 bp in length, with two major intergenic spacer sequences with 65.8% of base pairs of the entire spacer regions located between *trnQ* and *nad2* (83 bp), and *cox3* and *trnG* (38 bp).

In addition, 10 overlaps of 48 bp in length were present in the *B. macroscopa* mitogenome. The smallest was only 1 bp, and the largest was just 10 bp, located between *nad* and *trnH*.

The overall nucleotide composition of *B. macroscopa* mtDNA was 41.0% A, 39.9% T, 11.3% C, and 7.7% G. The A + T content was significantly biased (80.9%). The positive and negative skew

Table 2. Source and information for the phylogenetic analysis

Superfamily	Family	Species	Accession number	Length (bp)	References
Urodoidea	Urodidae	*Urodus decens*	KJ508062	15,279	Timmermans et al. (2014)
Yponomeutoidea	Plutellidae	*Plutella xylostella*	NC_025322	16,014	Dai et al. (2016)
	Yponomeutidae	*Prays oleae*	NC_025948	16,499	van Asch et al. (2016)
	Lyonetiidae	*L. malifoliella*	NC_018547	15,646	Wu et al. (2012)
Tineoidea	Tineidae	*Tineola bisselliella*	KJ508045	15,661	Timmermans et al. (2014)
Zygaenoidea	Zygaenidae	*Rhodopsona rubiginosa*	KM244668	15,248	Tang et al. (2014)
Tortricoidea	Tortricidae	*Adoxophyes honmai*	NC_008141	15,680	Lee et al. (2006)
		Grapholita dimorpha	NC_024582	15,813	Niu et al. (2016)
		Adoxophyes orana	NC_021396	15,343	Wu et al. (2013)
		Cydia pomonella	NC_020003	15,253	Shi et al. (2013)
		Rhyacionia leptotubula	NC_019619	15,877	Zhu et al. (2012)
		Grapholita molesta	NC_014806	15,717	Son and Kim (2011)
			HQ116416	15,776	Gong et al. (2012)
		Spilonota lechriaspis	NC_014294	15,368	Zhao et al. (2011)
		Choristoneura longicellana	NC_019996	15,759	Unpublished
		Acleris fimbriana	NC_018754	15,933	Zhao et al. (2014)
		Epiphyas postvittana	KJ508051	15,451	Timmermans et al. (2014)
		Retinia pseudotsugaicola	NC_022865	15,282	Unpublished
Gelechioidea	Oecophoridae	*E. sarcitrella*	KJ508037	15,317	Timmermans et al. (2014)
		P. suzukiella	KM875542	15,507	Park et al. (2014)
	Elachistidae	*E. eupostica*	KJ508047	15,347	Timmermans et al. (2014)
	Cosmopterigidae	*Perimede* sp.	KJ508041	15,131	Timmermans et al. (2014)
	Autostichidae	*O. novimundi*	KJ508036	15,408	Timmermans et al. (2014)
	Gelechiidae	*B. macroscopa*	KT354968	15,400	present study
Cossoidea	Cossidae	*Eogystia hippophaecolus*	NC_023936	15,431	Gong et al. (2013)
Hepialoidea	Hepialidae	*N. hunanensis*	NC_024424	15,301	Yi et al. (2016a)
		T. pui	NC_023530	15,064	Yi et al. (2016b)
		A. yunnanensis	NC_018095	15,816	Cao et al. (2012)
		T. renzhiensis	NC_018094	16,173	Cao et al. (2012)
Gracillarioidea	Gracillariidae	*Phyllonorycter platani*	KJ508044	15,791	Timmermans et al. (2014)
		Phyllonorycter froelichiella	KJ508048	15,538	Timmermans et al. (2014)
		Cameraria ohridella	KJ508042	15,513	Timmermans et al. (2014)
Copromorphoidea	Carposinidae	*Carposina sasakii*	NC_023212	15,611	Wu et al. (2016)

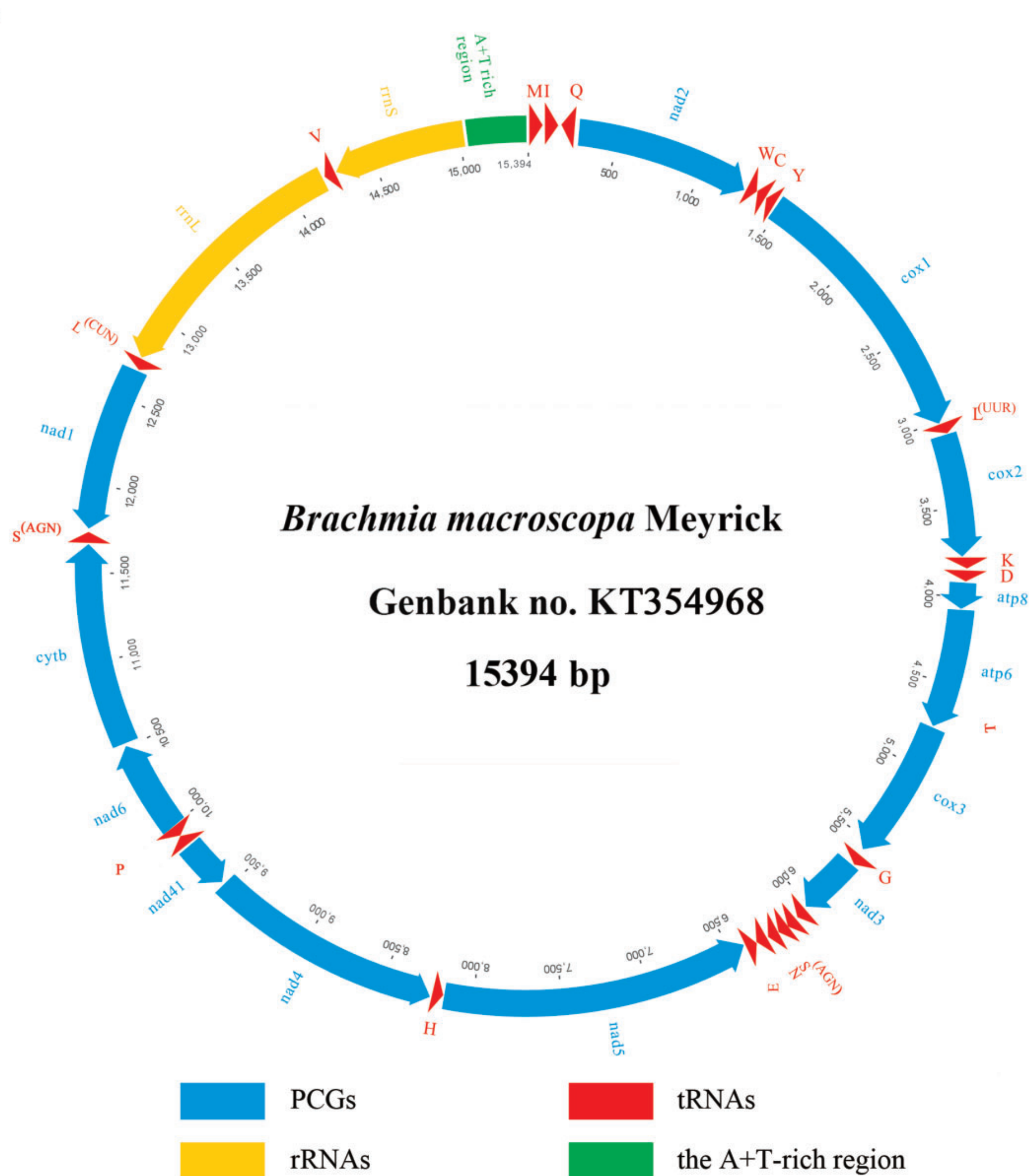

Fig. 1. Circular map of the *B. macroscopa* mitogenome. Annotations of the gene names were the standard abbreviations adopted in this article; single letters were drawn on the basis of IUPAC-IUB abbreviation for their matching amino acid.

were obviously distinct with an AT skew value of 0.014 and GC skew value of −0.188.

Codon Usage in PCGs

Regarding the PCGs, the major strand included *nad2, cox1, cox2, atp8, atp6, cox3, nad3, nad6*, and *cytb*, while the minor strand harbored *nad5, nad4, nad4L*, and *nad1*. Almost all of the PCGs started with ATN except for *cox1*, which started with CGA. Eleven of 13 PCGs ended with TAA, except for *cox2* with TA, and *nad4* with a single T.

The PCGs contained a total of 3,593 codons, excluding the start and termination codons. AUU (Ile), AAU (Asn), UUU (Phe), UUA (Leu), and AUA (Met) were the most abundant amino acid codons, and made up 47.1% of the total. The content of A + T was usually higher than G + C according to the summarized codon usage (Fig. 2A). The relative synonymous codon usage (RSCU) of the third position showed that the frequency of AU codons in two and four-fold degeneracy was greater than GC (Fig. 2B).

The Secondary Structure for RNA Genes

The two rRNA genes, 16 s (*rrnL*) and 12 s (*rrnS*), were 1,457 and 778 bp in length, respectively. The *rrnL* gene was situated in *trnS*$^{(UCN)}$ and *trnV*, and the *rrnS* gene was located between *trnV* and the A + T-rich region. Both of these genes were located on the

Table 3. Summary of the *B. macroscopa* mitogenome

Gene	Strand	Nucleotide no.	Size(bp)	IN	Anticodon	Start codon	Stop codon
trnM	J	1–67	67	8	CAT		
trnI	J	76–142	67	4	GAT		
trnQ	N	147–214	70	83	TTG		
nad2	J	298–1,284	987	−2		ATA	TAA
trnW	J	1,283–1,350	68	−8	TCA		
trnC	N	1,343–1,409	67	11	GCA		
trnY	N	1,421–1,489	69	3	GTA		
cox1	J	1,493–3,028	1536	−5		CGA	TAA
trnL$^{(UUR)}$	J	3,024–3,091	68	0	TAA		
cox2	J	3,092–3,778	687	−5		ATG	TA-
trnK	J	3,774–3,844	71	0	CTT		
trnD	J	3,845–3,913	69	9	GTC		
atp8	J	3,923–4,078	156	−7		ATA	TAA
atp6	J	4,072–4,749	678	−1		ATG	TAA
cox3	J	4,749–5,537	789	38		ATG	TAA
trnG	J	5,576–5,643	68	−3	TCC		
nad3	J	5,641–5,997	357	2		ATA	TAA
trnA	J	6,000–6,067	67	−1	TGC		
trnR	J	6,067–6,130	64	0	TCG		
trnN	J	6,131–6,196	66	3	GTT		
trnS$^{(AGN)}$	J	6,200–6,265	66	7	GCT		
trnE	J	6,273–6,335	63	1	TTC		
trnF	N	6,337–6,402	66	1	GAA		
nad5	N	6,404–8,146	1743	−10		ATT	TAA
trnH	N	8,137–8,201	65	0	GTG		
nad4	N	8,202–9,540	1339	0		ATG	T-
nad4L	N	9,541–9,834	294	2		ATG	TAA
trnT	J	9,837–9,902	66	0	TGT		
trnP	N	9,903–9,972	70	2	TGG		
nad6	J	9,975–10,502	528	14		ATA	TAA
cytb	J	10,517–11,671	1155	2		ATA	TAA
trnS$^{(UCN)}$	J	11,674–11,743	70	3	TGA		
nad1	N	11,747–12,703	957	−6		ATA	TAG
trnL$^{(CUN)}$	N	12,698–12,768	71	16	TAG		
rrnL	N	12,769–14,225	1457	0			
trnV	N	14,226–14,291	66	0	TAC		
rrnS	N	14,292–15,069	778	0			
A + T-rich	–	15,070–15,394	325	0			

N-strand. The complete secondary structures of the *rrnL* and *rrnS* molecules were determined and are shown in Figs. 3 and 4, respectively. The *rrnL* secondary structure contained six domains, made up of two conserved and four variable regions, and three other domains appeared in the *rrnS* secondary structure. Twenty-two tRNA genes, ranging from 63 to 71 bp in length, were contained in the *B. macroscopa* mitogenome. Fourteen of these genes were mapped on the J-strand and eight were mapped on the N-strand. Typical cloverleaf secondary structures were also found in this species, except for *trnS*$^{(UCN)}$ which lacked a dihydrouridine arm (Fig. 5).

Features in the A + T-Rich Region

The A + T-rich region, located between the *trnM* and *rrnS* genes, was 325 bp long with a higher content (96.7%) and the absence of a large repeating fragment. The conserved structure consisted of an 'ATAGT'-like motif, and included a poly-T. The poly-T was considered to be the origin of the minor strand replication (Fig. 6A). Three microsatellites, '$(TA)_8$', '$(TA)_9$', and '$(TA)_7$', were observed in this region, and they were located 205, 241, and 261 bp upstream of *rrnS*, respectively. The conserved 'ATTTA' sequence was located between the microsatellite $(TA)_8$ and $(TA)_9$. A potential stem-loop structure existed in the A + T-rich region without the 'TATA' sequence at the 5′ end and without the 'G(A)nT' at the 3′ end (Fig. 6B).

Phylogenetic Relationships

The phylogenetic relationships among the superfamilies within the Ditrysia were reconstructed and are shown in Figs. 7 and 8. The topological structures of the two trees were almost identical. The ditrysian group was supported as a monophyletic group by a high bootstrap value of 100% and a posterior probability of 1.00. The results from both ML and BI analysis show that the Tortricoidea is a robust monophyletic group supported by a high bootstrap value of 100% and is a sister to the clade ((Urodoidea + Copromorphoidea) + (Gelechioidea + (Cossoidea + Zygaenidae))) supported by a bootstrap value of 100% and a posterior probability of 1.00. However, bootstrap values of all small clades within the latter clade are lower. These six superfamilies are, together, a sister of the clade (Yponomeutoide +Gracillarioidea), in which Gracillarioidea is a robust monophyletic group, whereas Yponomeutoide is a

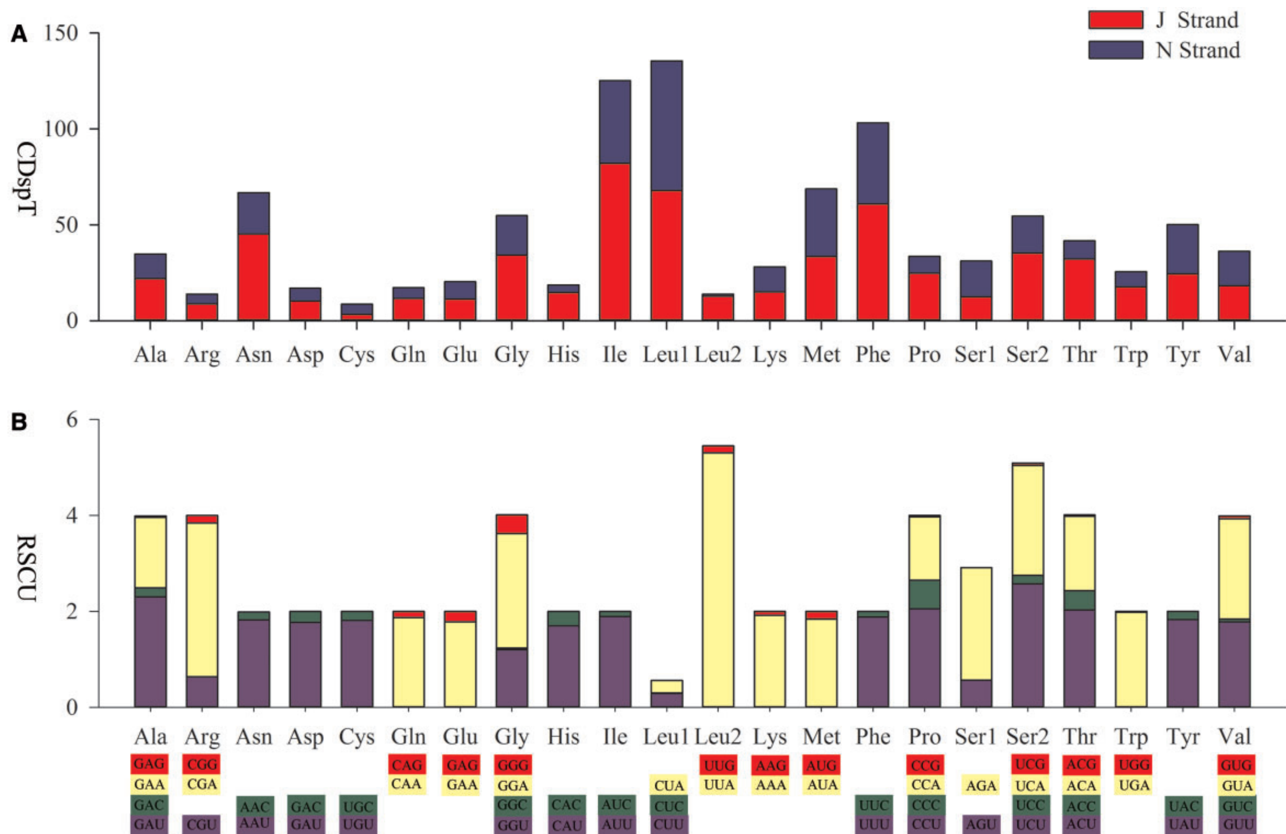

Fig. 2. Codon usage in the *B. macroscopa* mitogenome. (A) CDspT (codons per thousand) indicate the codons used in coding amino acids per thousand codons. Codon families are given on the *x*-axis. (B) RSCU.

polyphyletic group. Tortricoidea were recovered as a sister to the rest of Apoditrysia in the analyses, and this was supported by a bootstrap value of 100% and a posterior probability of 1.00. The Gelechioidea was assumed be a sister group to the Apoditrysia (Cho et al. 2011), but it was not supported in our results because the Gelechioidea was nested within the clade Apoditrysia in the two trees. The Apoditrysia group (Gelechioidea, Zygaenoidea, Cossoidea, Copromorphoidea, Urodoidea, and Tortricoidea) was a monophyletic clade with a bootstrap value of 100% and a posterior probability of 1.00. A sister relationship between Yponomeutoidea + Gracillarioidea and the Apoditrysia was supported by a bootstrap value of 88% and a posterior probability of 1.00. The superfamily Tineoidea was a monophyletic clade with a bootstrap value of 100% and a posterior probability of 1.00. This is the earliest clade derived from Ditrysia, supporting the general agreement that tineoids are the oldest ditrysian superfamily (Minet 1991, Kristensen and Skalski 1998). The superfamily Gelechioidea, which includes *O. novimundi*, *E. sarcitrella*, *E. eupostica*, *B. macroscopa*, and *Perimede* sp. in this study, formed a monophyletic group supported by a posterior probability of 1.00 (Fig. 8).

Discussion

Genome Organization and Base Characteristics

The gene order and orientation of the *B. macroscopa* mitogenome were identical to the fully sequenced ditrysian species with the gene order *trnM-trnI-trnQ*. Nevertheless, it was different from the gene order *trnI-trnQ-trnM* in the non-ditrysian lineage of Lepidoptera (Flook et al. 1995, Yi et al. 2014).

The AT nucleotides in the *B. macroscopa* mitogenome skewed slightly, and the content was 81.0%, which is the same as other lepidopteran insects in the region; such as, *Lobocla bifasciatus, Argynis nerippe* (Kim et al. 2011, 2014), and *Argynnis hyperbius* (Wang et al. 2012). The AT skew was 0.013, which indicated the occurrence of A more than T. The AT content in the PCGs was 79.2%, which is similar to that of *Potanthus flavus* (Kim et al. 2014) and *Kallima inachus* (Qin et al. 2012). The AT content of *cox1* was the lowest, at 72.1%, whereas *atp8* was significantly higher, at 95.5%, among the PCGs. Numerous studies have come to the conclusion that there is usually more A than T, and more C than G on the J strand, but the situation might occasionally be reversed for some species (Wei et al. 2010). We, however, observed that PCGs on the J-strand had a positive AT-skew and GC-skew. That means that this species has more A than T, and less C than G on the major strand. This novel observation had never before been made in the mitogenome of any insect. Regrettably, we have not elucidated the mechanism of this phenomenon. Nevertheless, early studies found that the value of the GC skew was not associated with gene direction but with replication orientation, while the value of the AT skew could change over gene direction, replication, and codon positions (Wei et al. 2010).

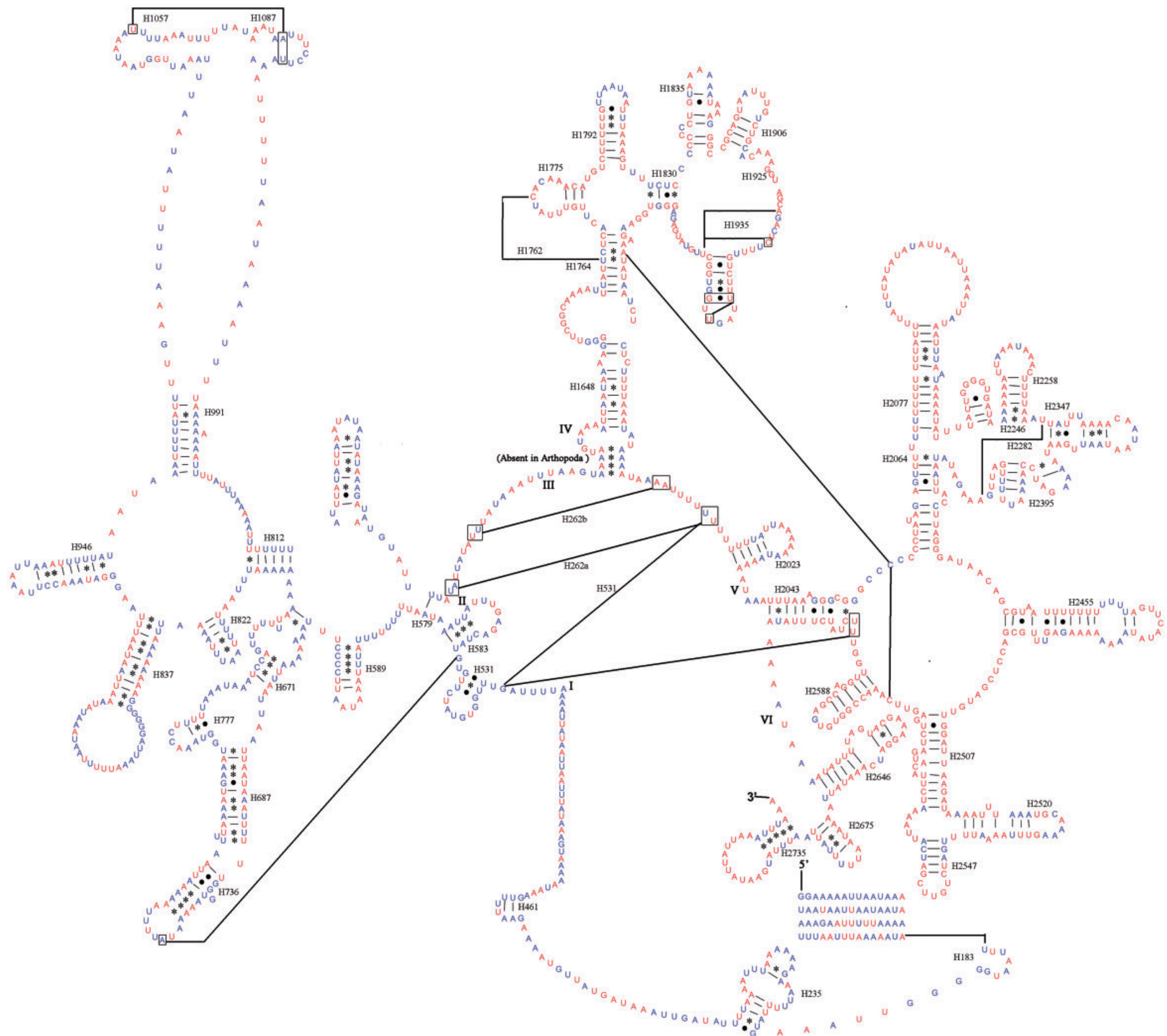

Fig. 3. Predicted secondary structure of the *rrnL* gene in the *B. macroscopa* mitogenome. Red-colored letters stand for variable positions and blue for conserved positions. Tertiary structures are noted by continuous lines. Base-pairing is delineated as follows: Watson-Crick pairs are joined by lines, GU pairs by dots, and other non-canonical base pairs by asterisks. Each domain is indicated with Roman numerals.

Intergenic Spacer Regions and Overlapping

The spacer is located commonly between *trnQ* and *nad2*, but it is not conserved, as its length varies in lepidopteran species. It was the longest of all spacers of *B. macroscopa*. This result might support the conclusion that the spacer between *trnQ* and *nad2* has no functional significance or that it acts as another origin of replication (Cameron and Whiting 2008). In addition, the spacer, which has only been shown in the lepidopteran insects, had high homology with adjacent *nad2* genes (Xia et al. 2011).

The common overlap (7 bp), between *atp8* and *atp6* that is widely distributed in other lepidopteran mitogenomes (Jiang et al. 2009, Zhu et al. 2013, Chen et al. 2014), was also present in the *B. macroscopa* mitogenome. Meanwhile, the position of the maximum overlap between *nad5* and *trnH* was different from other lepidopteran mitogenomes, such as *Diaphania pyloalis*, which has the maximum overlap between *trnF* and *nad5* (Zhu et al. 2013), *Chilo suppressalis*, which also has the maximum overlap between *trnF* and *nad5* (Chai et al. 2012), and *Attacus atlas*, which has the maximum overlap between *trnW* and *trnC* (Chen et al. 2014).

Protein-Coding Genes

'ATA' and 'ATG' were present as start codons with the same frequency among the 12 PCGs, while 'CGA' was the start codon of *cox1*. The sequencing of the 5′ region of *cox1* genes from 39 lepidopteran species indicated the conservation of 'CGA' in lepidopteran mitogenomes and implied that 'CGA' may be a synapomorphic feature in Lepidoptera (Kim et al. 2009).

Eleven of the 13 PCGs ended with 'TAA' codons, while the remaining two stopped with 'TTA' or a single 'T'. The incomplete

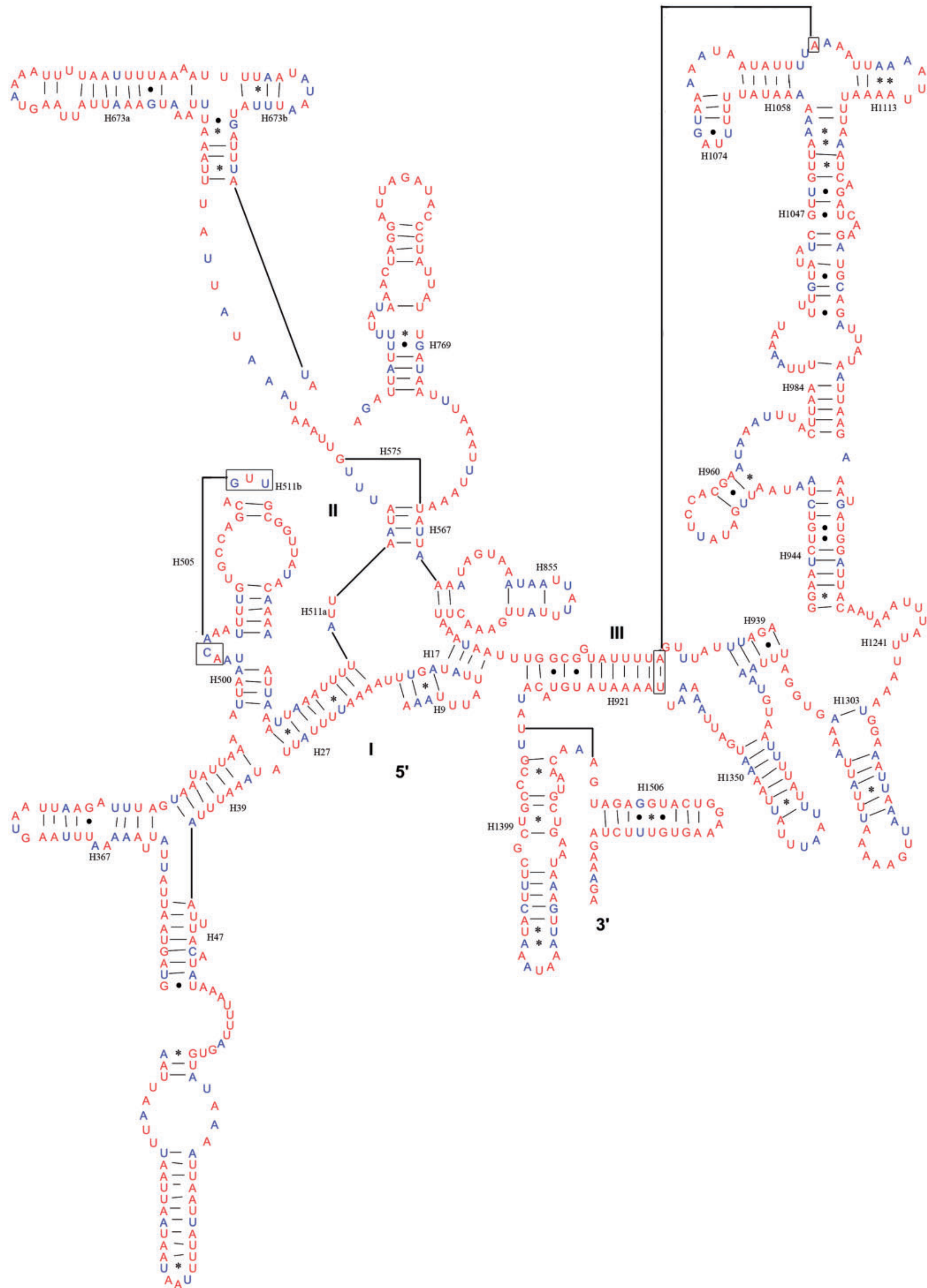

Fig. 4. Predicted secondary structure of the *rrnS* gene in the *B. macroscopa* mitogenome. Red-colored letters stand for variable positions and blue for conserved positions. Tertiary structures are noted by continuous lines. Base-pairing is delineated as follows: Watson-Crick pairs are joined by lines, GU pairs by dots, and other non-canonical base pairs by asterisks. Each domain is indicated with Roman numerals.

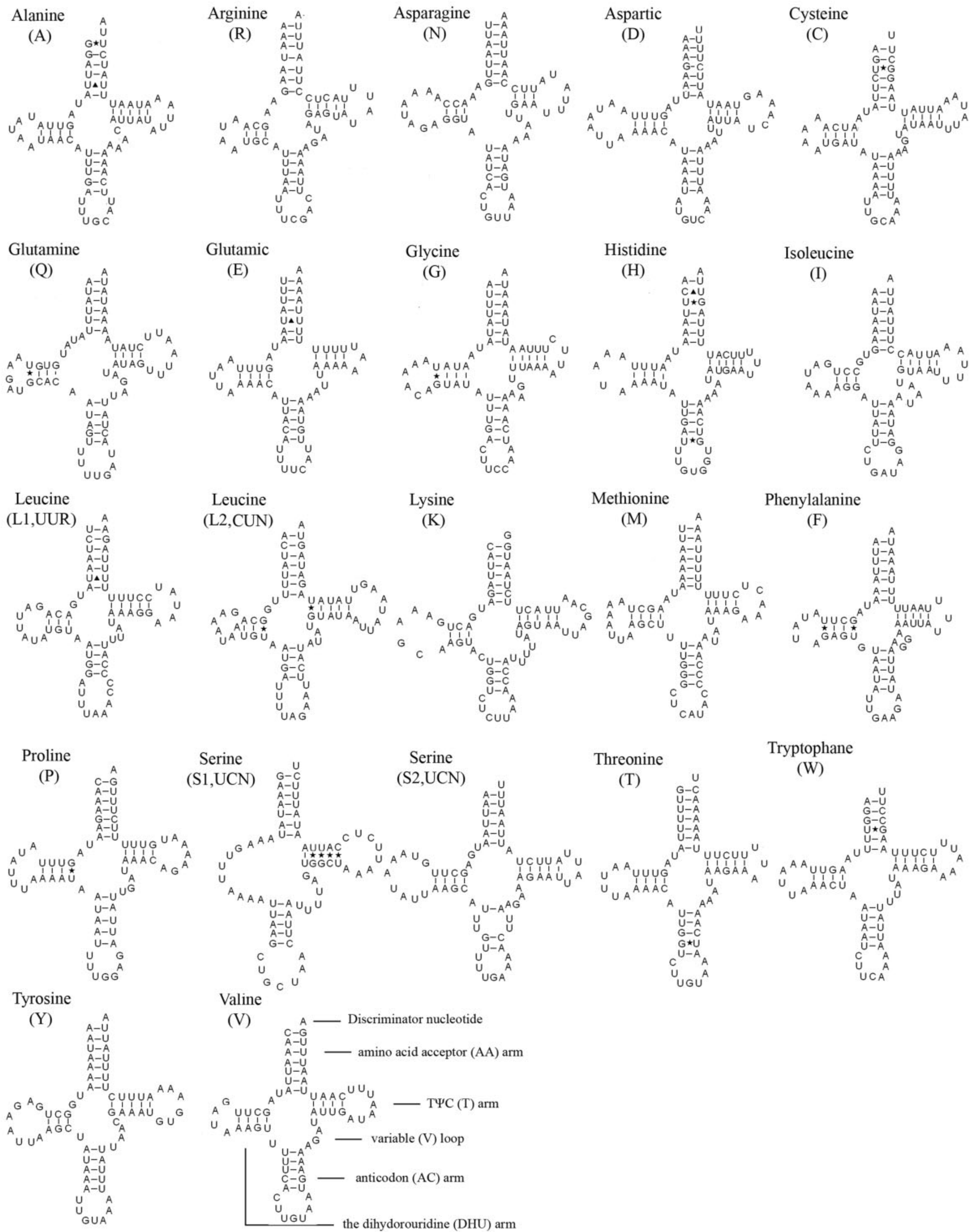

Fig. 5. Predicted secondary structure of tRNAs gene in the *B. macroscopa* mitogenome. Dashes (–) indicate Watson-Crick base-pairing, centered solid pentagram (★) indicate G-U base-pairing, and solid triangles (▲) indicate mismatches.

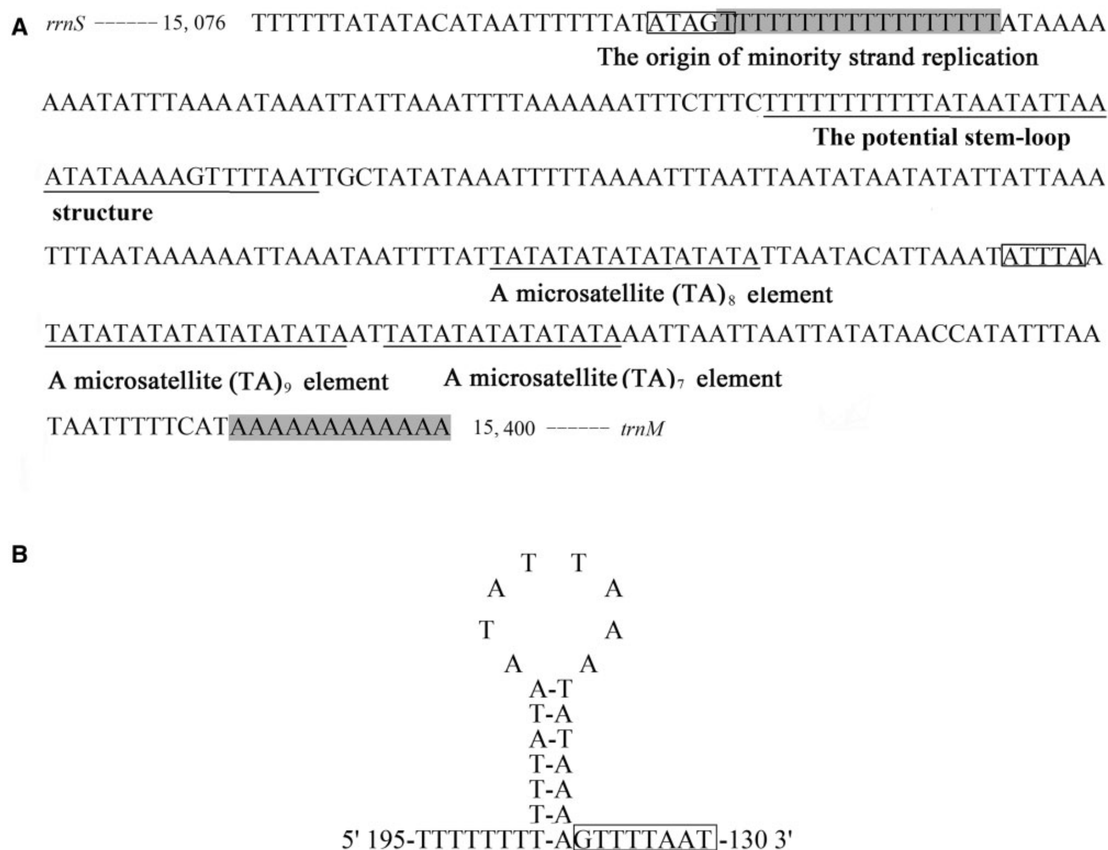

Fig. 6. The A + T-rich region of *B. macroscopa* mitogenome. (A) Structure of the A + T-rich region. (B) A potential stem-loop structure found in the A + T-rich region.

termination codons could be completed by the mRNA process of polyadenylation (Anderson et al. 1981). It is important that the incomplete stop codons could make up the complete TAA in the assembly process of mRNA (Boore 1999, Gong et al. 2012). It was concluded that A and U were more frequently used in PCGs because the value for the RSCU of NNU and NNA codons was always >1.

rRNA and tRNA Genes

The rRNA genes of insects are usually conserved in mitogenomes. Therefore, the secondary structures for *rrnL* and *rrnS* RNA in *B. macroscopa* are similar to those of other Lepidoptera (Gillespie et al. 2006, Niehuis et al. 2006a, b; Chai et al. 2012). The base pairs in the *rrnL* gene did not fully comply with Watson-Crick base-pairing. The mispairing of H991 in the *rrnL* secondary structures was not observed in *Leucoptera malifoliella* (Lepidoptera: Lyonetiidae) (Fig. 4), but it is very common in lepidopteran insects; such as, *Apocheima cinerarius* (Lepidoptera: Geometridae), *C. suppressalis* (Lepidoptera: Pyralidae), *Manduca sexta* (Lepidoptera: Sphingidae), and *Zygaena sarpedon lusitanica* (Lepidoptera: Zygaenidae). The H47, H673, H1047, and H1241 in the secondary structure of the *rrnS* gene were different from those of *M. sexta* in structure and length. The H47 portion was one of the variable sites among the species (Gillespie et al. 2006, Gong et al. 2012); therefore, it could provide valuable information on the phylogenetic relationship of H39 and H367 (Wei et al. 2010). The majority of the tRNA is likely to fold into an iconic clover-leaf secondary structure, except for $trnS^{(UCN)}$. The $trnS^{(UCN)}$ was the only special case that could not form a complete cloverleaf structure, but the incomplete structure evolved early in the metazoans (Garey and Wolstenholme 1989, Wolstenholme 1992). However, previous research has shown that the $trnS^{(UCN)}$ is highly conserved in nearly all families, as well as in *B. macroscopa*.

Phylogenetic Relationships

Knowledge of insects involves insect ecology, behavior, systematics, host plant choices, and so on, but information about the worldwide superfamily Gelechioidea has been very limited (Hodges 1998). The sister group of Gelechioidea is still unknown (Mutanen et al. 2010). Mitogenomic phylogeny research was carried out by Timmermans et al. (2014), and it demonstrated that the Gelechioidea was not grouped with other lower Apoditrysia. The phylogenetic systematics of 10 superfamilies in Lepidoptera were constructed based on mitochondrial data that showed a sister relationship between Gelechioidea and Zygaenidae + Cossoidea, because both of them cluster in a same node and have a positive Bayesian posterior probability of 1.00. Significantly, our study may support the Gelechioidea belonging to the Apoditrysia, and it differed from the findings of Timmermans et al. (2014). The two analyses were both conducted based on mitogenome data, and whether these differences were due to the introduction of the

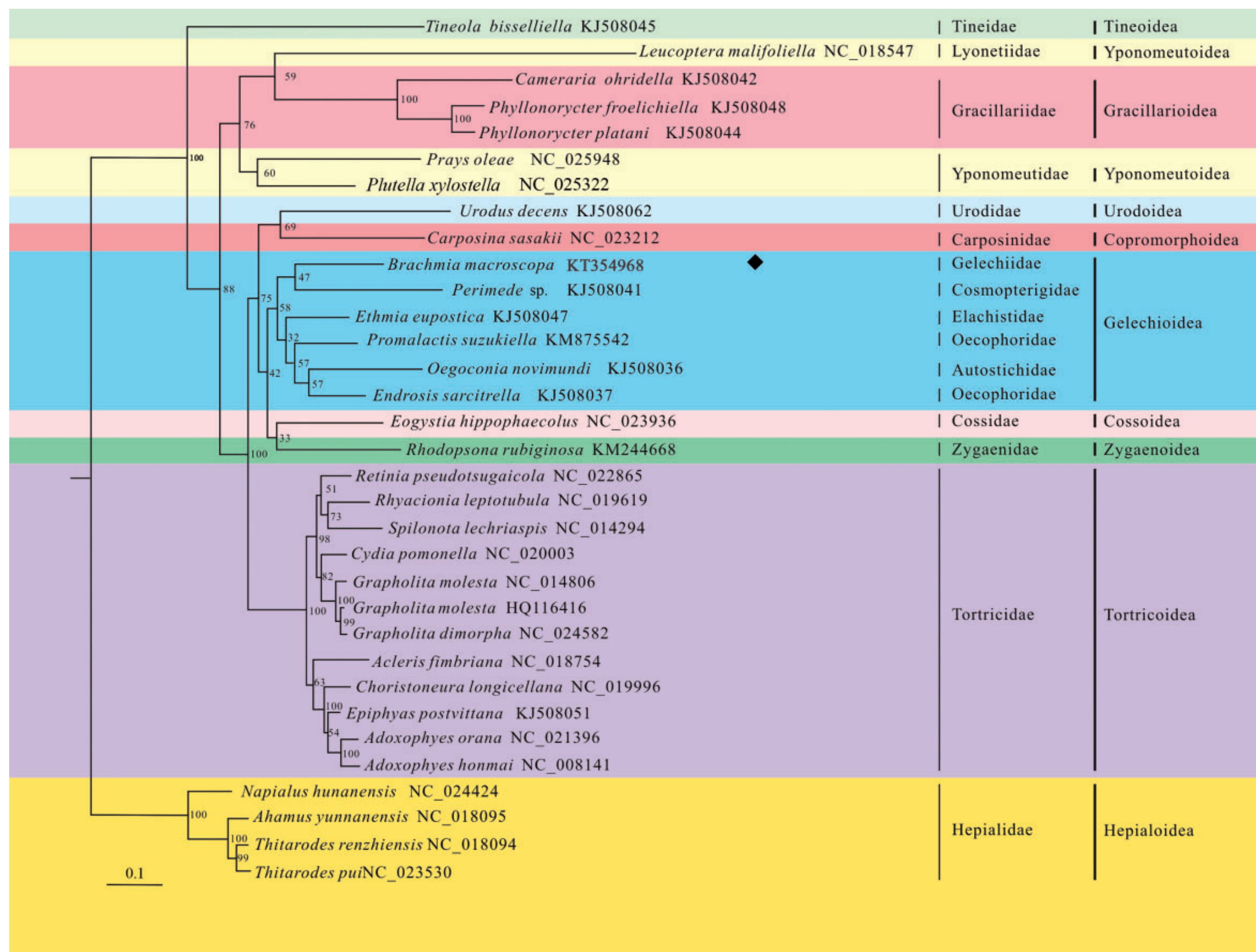

Fig. 7. Phylogeny of lepidopteran superfamilies including *B. macroscopa*. ML phylogram using the concatenated 13 PCGs of mitogenomes obtained in these species, which used partitioned models for analysis. The scale bar indicates the number of substitutions per spot, and values of each node specify bootstrap percentages of 1,000 replicates.

complete mitogenome in this study remains to be investigated. The phylogenetic relationship among the Yponomeutoide, Gracillarioidea, Gelechioidea, and Apoditrysia were not well resolved by Kristensen et al. (2007). In our results, the Yponomeutoide + Gracillarioidea and Apoditrysia were sister groups and clustered on the same branch, with a bootstrap value of 88% and a high posterior probability of 1.00. Although the value of ML was lower, it can still provide some information for the uniform relationships. The structure was supported by Regier et al. (2013), and it had many differences from the nested Yponomeutoide + Gracillarioidea and Gelechioidea (Kristensen and Skalski 1998).

The ML and BI analyses have different advantages in reconstructing phylogenetic trees, and in some instances the results may be different according to the different methods. The results of this study, which revealed the monophyly of Gelechioidea, was mostly supported by previous analyses that were based on morphology, ecology, and mitogenomes (Passoa 1995, Kaila 2004, Bucheli and Wenzel 2005, Regier et al. 2009, Mutanen et al. 2010, Kaila et al. 2011). Although the ML value was lower, the two separate analyses correctly interpreted the monophyly of Gelechioidea. Six species were chosen, representing five subfamilies (Symmocinae, Oecophoninae, Ethmiinae, Dichomeridinae, and Chrysopeleiinae). According to the analysis of Mutanen et al. (2010), Autostichidae was not monophyletic unless Glyphydoceridae and Deoclonidae were included. The tree, in Figs. 7 and 8, showed that Autostichidae and Oecophoridae clustered in the same node, and therefore the monophyly of Autostichidae was not confirmed. With further observations, the Ethmiinae and the other two subfamilies had higher bootstrap values and strongly resembled the earlier results of phylog enetic relationships indicated by Passoa (1995) and Bucheli and Wenzel (2005). The consensus on familial relationships ((Autostichidae + Oecophoridae) + Elachistidae) + (Gelechiidae + Cosmopterigidae) by ML and ((Elachistidae + Gelechiidae) + Oecophoridae) + (Autostichidae + Cosmopterigidae) by BI obviously conflicted with the latest molecular analysis that revealed different affinities (Elachistidae + (Cosmopterigidae + Gelechiidae) + (Autostichidae + Oecophoridae)) (Heikkilä et al. 2014). The challenge of getting better mitogenome-based phylogenies probably requires that more Gelechioidea mitogenomes are sequenced.

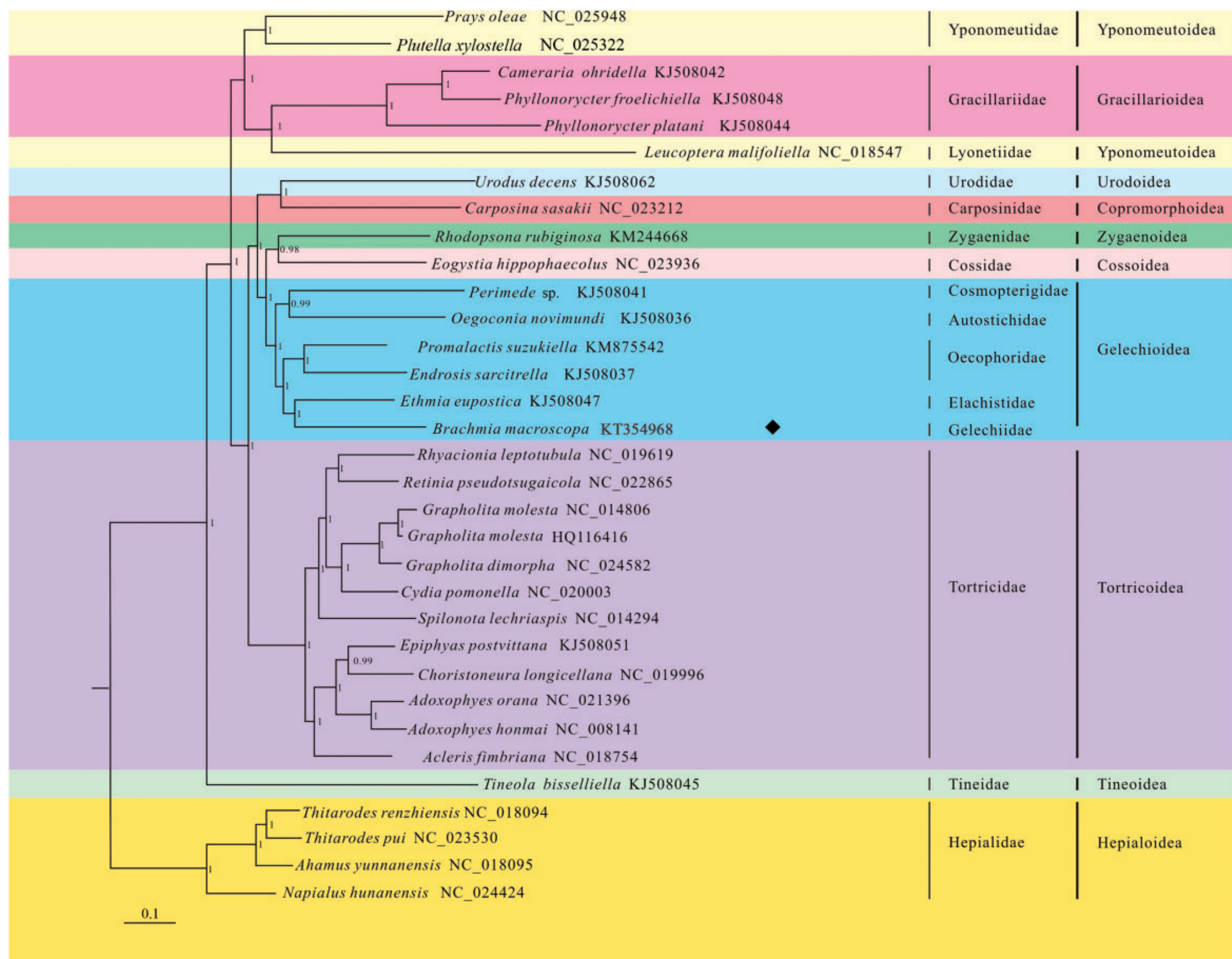

Fig. 8. BI phylogram using the concatenated 13 PCGs of mitogenomes obtained in these species, which used partitioned models for analysis. The scale bar indicates the number of substitutions per spot, and values of each node specify bootstrap percentages of 1,000 replicates.

Acknowledgments

We acknowledge the help of Jie Zhu and Zi-Shu Chen for providing much guidance for using the software, both for analysis and presentation. Dr. Layne J. Westover kindly suggested improvements to this article and corrected the language of the manuscript. This study was supported by the National Major Science and Technology Project of the Twelfth Five-year Plans (2012BAD27B00), Scientific Research Fund of Hunan Provincial Education Department (12A070), and the Hunan Provincial Natural Science Foundation for Distinguished Young Scholar of China (14JJ1023).

References Cited

Abascal, F., R. Zardoya, and D. Posada. 2005. Protest: selection of best-fit models of protein evolution. Bioinformatics 21: 2104–2105.

Anderson, S., A. T. Bankier, B. G. Barrell, M. H. L. Debruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, et al. 1981. Sequence and organization of the human mitochonadrial genome. Nature 290: 457–465.

Avise, J. C. 2000. Phylogeography, the history and formation of species. Harvard University Press, Cambridge, MA.

Bernt, M., A. Donath, F. Jühling, F. Externbrink, C. Florentz, G. Fritzsch, J. Pütz, M. Middendorf, and P. F. Stadler. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol. Phylogenet. Evol. 69: 313–319.

Boore, J. L. 1999. Animal mitochondrial genomes. Nucleic Acids Res. 27: 1767–1780.

Bucheli, S. R., and J. Wenzel. 2005. Gelechioidea (Insecta: Lepidoptera) systematics: a reexamination using combined morphology and mitochondrial DNA data. Mol. Phylogenet. Evol. 35: 380–394.

Cameron, S. L. 2014. How to sequence and annotate insect mitochondrial genomes for systematic and comparative genomics research. Syst. Entomol. 39: 400–411.

Cameron, S. L., and M. F. Whiting. 2008. 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 408: 112–123.

Cameron, S. L., C. Lambkin, S. C. Barker, and M. F. Whiting. 2007. Amitochondrial genome phylogeny of Diptera: whole genome sequence data accurately resolve relationships over broad timescales with high precision. Syst. Entomol. 32: 40–59.

Cao, Y. Q., C. Ma, J. Y. Chen, and D. R. Yang. 2012. The complete mitochonadrial genomes of two ghost moths, *Thitar odes renzhiensis* and *Thitarodes yunnanensis*: the ancestral gene arrangement in Lepidoptera. BMC Genomics 13: 276.

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17: 540–552.

Chai, H. N., Y. Z. Du, and B. P. Zhai. 2012. Characterization of the complete mitochondrial genomes of *Cnaphalocrocis medinalis* and *Chilo suppressalis* (Lepidoptera: Pyralidae). Int. J. Biol. Sci. 8: 561.

Chen, M. M., Y. Li, M. Chen, H. Wang, Q. Li, R. X. Xia, C. Y. Zeng, Y. P. Li, Y. Q. Liu, and L. Qin. 2014. Complete mitochonadrial genome of the atlas moth, *Attacus atlas* (Lepidoptera: Saturniidae) and the phylogenetic relationship of Saturniidae species. Gene 545: 95–101.

Cho, S. L., A. Zwick, J. C. Regier, C. Mitter, M. P. Cummings, J. Yao, Z. Du, H. Zhao, A. Y. Kawahara, S. Weller, et al. 2011. Can deliberately incomplete gene sample augmentation improve a phylogeny estimate for the advanced moths and butterflies (Hexapoda: Lepidoptera)? Syst. Biol. 60: 782–796.

Dai, L. S., B. J. Zhu, C. Qian, C. F. Zhang, J. Li, L. Wang, G. Q. Wei, and C. L. Liu. 2016. The complete mitochondrial genome of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Mitochondr. DNA 27: 1512–1513.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9: 772.

Drummond, A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, J. Heled, M. Kearse, R. Moir, S. Stones-Havas, S. Sturrock, et al. 2010. Geneious version 4.8.4, created by Biomatters.

Flook, P. K., C.H.F. Rowell, and G. Gellissen. 1995. The sequence, organization, and evolution of the *Locusta migratoria* mitochondrial genome. J. Mol. Evol. 41: 928–941. Available from <http://www.geneious.com/>.

Gillespie, J. J., J. S. Johnston, J. J. Cannone, and R. R. Gutell. 2006. Characteristics of the nuclear (18S, 58S, 28S and 5S) and mitochondrial (12S and 16S) rRNA genes of *Apis mellifera* (Insecta: Hymenoptera): structure, organization, and retrotransposable elements. Insect Mol. Biol. 15: 657–686.

Garey, J. R., and D. R. Wolstenholme. 1989. Platyhelminth mitochondrial DNA: evidence for early evolutionary origin of a $tRNAser^{AGN}$ that contains a dihydrouridine arm replacement loop, and of serine-specifying AGA and AGG codons. J. Mol. Evol. 28: 374–387.

Gong, Y. J., B. C. Shi, Z. J. Kang, F. Zhang, and S. J. Wei. 2012. The complete mitochondrial genome of the oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae). Mol. Biol. Rep. 39: 2893–2900.

Gong, Y. J., Q. L. Wu, and S. J. Wei. 2013. The first complete mitogenome for the superfamily Cossoidea of Lepidoptera: the seabuckthorn carpenter moth *Eogystia hippophaecolus*. Mitochondr. DNA 25: 288–289.

Heikkilä, M., M. Mutanen, M. Kekkonen, and L. Kaila. 2014. Morphology reinforces proposed molecular phylogenetic affinities: a revised classification for Gelechioidea (Lepidoptera). Cladistics 30: 563–589.

Hodges, R. W. 1998. The Gelechioidea. pp, 132–158. *In* N.P. Kristensen (ed.), Handbook of zoology, vol. IV, Arthropoda: Insecta, part 35, Lepidoptera, moths and butterflies, vol. 1. Walter de Gruyter, Berlin.

Huang, G. H., and J. H. Li. (eds.) 2013**.** Color handbook of in insect pests of aquatic vegetables in China, pp, 85–87. Hubei Publishers of Science and Technology, Wuhan

Inohira, K., T. Hara, and E. T. Matsuura. 1997. Nucleotide sequence divergence in the A + T rich region of mitochondrial DNA in *Drosophila simulans* and *Drosophila mauritiana*. Mol. Biol. Evol. 14: 814–822.

Jiang, S. T., G. Y. Hong, M. Yu, N. Li, Y. Yang, Y. Q. Liu, and Z. J. Wei. 2009. Characterization of the complete mitochondrial genome of the giant silkworm moth, *Eriogyna pyretorum* (Lepidoptera: Saturniidae). Int. J. Biol. Sci. 5: 351–365.

Junqueira, A. C., A. C. Lessinger, T. T. Torres, F. R. da Silvab, A. L. Vettorec, P. Arruda, and A. M. Azeredo Espin. 2004. The mitochondrial genome of the blowfly *Chrysomya chloropyga* (Diptera: Calliphoridae). Gene 339: 7–15.

Kaila, L. 2004. Phylogeny of the superfamily Gelechioidea (Lepidoptera: Ditrysia): an exemplar approach. Cladistics 20: 303–340.

Kaila, L., M. Mutanen, and T. Nyman. 2011. Phylogeny of the mega-diverse Gelechioidea (Lepidoptera): adaptations and determinants of success. Mol. Phylogenet. Evol. 61: 801–809.

Kim, M. I., J. Y. Baek, M. J. Kim, H. C. Jeong, K. G. Kim, C. H. Bae, Y. S. Han, B. R. Jin, and I. Kim. 2009. Complete nucleotide sequence and organization of the mitogenome of the red-spotted apollo butterfly, *Parnassius bremeri* (Lepidoptera: Papilionidae) and comparison with other lepidopteran insects. Mol. Cells 28: 347–363.

Kim, M. J., A. R. Wang, J. S. Park, and I. Kim. 2014. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperiidae) and phylogenetic reconstruction of Lepidoptera. Gene 549: 97–112.

Kim, M. J., H. C. Jeong, S. R. Kim, and I. Kim. 2011. Complete mitochondrial genome of the *nerippe* fritillary butterfly, *Argynnis nerippe* (Lepidoptera: Nymphalidae). Mitochondr. DNA 22: 86–88.

Kristensen, N. P., and A. W. Skalski. 1998. Phylogeny and palaeontology, Pp. 7–25. *In* N.P. Kristensen (ed.), Handbook of zoology, vol. IV, Arthropoda: Insecta, part 35, Lepidoptera, moths and butterflies, vol. 1. Walter de Gruyter, Berlin.

Kristensen, N. P., M. J. Scoble, and O.L.E. Karsholt. 2007. Lepidoptera phylogeny and systematics: the state of inventorying moth and butterfly diversity. Zootaxa 1668: 699–747.

Lee, E. S., K. S. Shin, M. S. Kim, H. Park, S. Cho, and C. B. Kim. 2006. The mitochondrial genome of the smaller tea tortrix *Adoxophyes honmai* (Lepidoptera: Tortricidae). Gene 373: 52–57.

Lewis, J. A., A. Huq, W. Liu, and A. Jacob. 1995. Induction of antiviral responses and gene activation by intracellular interferon. Virology 212: 438–450.

Lowe, T. M., and S. R. Eddy. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 5: 955–964.

Minet, J. 1991. Tentative reconstruction of the ditrysian phylogeny (Lepidoptera: Glossata). Insect Syst. Evol. 22: 69–95.

Mutanen, M., N. Wahlberg, and L. Kaila. 2010. Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. Proc. R. Soc. B. 277: 2839–2848.

Niehuis, O., C. M. Naumann, and B. Misof. 2006a. Identification of evolutionary conserved structural elements in the mt SSU rRNA of Zygaenoidea (Lepidoptera): a comparative sequence analysis. Org. Divers. Evol. 6: 17–32.

Niehuis, O., S. H. Yen, C. M. Naumann, and B. Misof. 2006b. Higher phylogeny of zygaenid moths (Insecta: Lepidoptera) inferred from nuclear and mitochondrial sequence data and the evolution of larval cuticular cavities for chemical defence. Mol. Phylogenet. Evol. 39: 812–829.

Niu, F. F., X. L. Fan, and S. J. Wei. 2016. Complete mitochondrial genome of the *Grapholita dimorpha* Komai (Lepidoptera: Tortricidae). Mitochondr. DNA 27: 775–776.

Park, J. S., S. S. Kim, K. Y. Kim, and K. Iksoo. 2014. Complete mitochondrial genome of Suzuki's Promolactis moth *Promalactis suzukiella* (Lepidoptera: Oecophoridae). Mitochondr. DNA Published online 12 November 2014 DOI:10.3109/19401736.2014.982572.

Passoa, S. C. 1995. Larval and pupal systematics of Nearctic Amphisbatinae and Depressariinae (Lepidoptera: Oecophoridae). University of Illinois at Urbana-Champaign.

Qin, X. M., Q. X. Guan, D. L. Zeng, F. Qin, and H. M. Li. 2012. Complete mitochondrial genome of *Kallima inachus* (Lepidoptera: Nymphalidae: Nymphalinae): comparison of *K. inachus* and *Argynnis hyperbius*. Mitochondr. DNA 23: 318–320.

Regier, J. C., C. Mitter, A. Zwick, A. L. Bazinet, M. P. Cummings, A. Y. Kawahara, J. C. Sohn, D. J. Zwickl, S. Cho, D. R. Davis, et al. 2013. A large-scale, higher-level, molecular phylogenetic study of the insect order Lepidoptera (moths and butterflies). PLoS One 8: e58568.

Regier, J. C., A. Zwick, M. P. Cummings, A. Y. Kawahara, S. Cho, S. Weller, A. Roe, J. Baixeras, J. W. Brown, C. Parr, et al. 2009. Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. BMC Evol. Biol. 9: 280.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference unader mixed models. Bioinformatics 19: 1572–1574.

Shao, R., N. J. Campbell, and S. C. Barker. 2001. Numerous gene rearrangements in the mitochondrial genome of the wallaby louse, *Heterodoxus macropus* (Phthiraptera). Mol. Biol. Evol. 18: 858–865.

Shi, B. C., W. Liu, and S. J. Wei. 2013. The complete mitochondrial genome of the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). Mitochondr. DNA 24: 37–39.

Simon, C., T. R. Buckley, F. Frati, J. B. Stewart, A. T. Beckenbach, and C. B. Kim. 2006. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 37: 545–579.

Simon, C., F. Frati, A. Beckenbach, C. Bernie, L. Hong, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene

sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.

Son, Y., and Y. Kim. 2011. The complete mitochondrial genome of *Grapholita molesta* (Lepidoptera: Tortricidae). Ann. Entomol. Soc. Am. 104: 788–799.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313.

Taanman, J. W. 1999. Themitochondrial genome: structure, transcription, translation and replication. Biochim Biophys Acta Bioenerg 1410: 103–123.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30: 2725–2729.

Tang, M., M. Tan, G. Meng, S. Yang, X. Su, S. Liu, W. Song, Y. Li, Q. Wu, A. Zhang, et al. 2014. Multiplex sequencing of pooled mitochondrial genomes—a crucial step toward biodiversity analysis using mito-metagenomics. Nucleic Acids Res. 42: e166.

Timmermans, M. J., D. C. Lees, and T. J. Simonsen. 2014. Towards a mitogenomic phylogeny of Lepidoptera. Mol. Phylogenet. Evol. 79: 169–178.

van Asch, B., I. Blibech, I. Pereira-Castro, F. T. Rei, and L. T. da Costa. 2016. The mitochondrial genome of *Prays oleae* (Insecta: Lepidoptera: Praydidae). Mitochondr. Published online 25 November 2014 DOI:10.3109/19401736.2014.982579.

Wang, J. L., and R. R. Tan. 2011. Effect of temperature developmental duration and feeding amount and in *Brachimia macroscopa* Meyrick. Changjiang Veget. 4: 75–77.

Wang, X. C., X. Y. Sun, Q. Q. Sun, D. X. Zhang, J. Hu, Q. Yang, and J. S. Hao. 2012. The complete mitochondrial genome of the laced fritillary *Argyreus hyperbius* (Lepidoptera: Nymphalidae). Zool. Res. 32: 465–475.

Wei, S., M. Shi, M. J. Sharkey, C. Achterberg, and X. X. Chen. 2010. Comparative mitogenomics of Braconidae (Insecta: Hymenoptera) and the phylogenetic utility of mitochondrial genomes with special reference to Holometabolous insects. BMC Genomics 11: 371.

Wei, S. J., M. Shi, X. X. Chen, M. J., Sharkey, van C., Achterberg, G. Y. Ye, and J. H. He. 2010. New views on strand asymmetry in insect mitochondrial genomes. PLoS One 5: e12708.

Wen, L., and C. C. Wang. 2010. Biological features, occurrence regulation and control technology of *Brachmia macroscopa* Meyrick (Lepidoptera: Gelechiidae). China Veget. 13: 28–29.

Wolstenholme, D. R. 1992. Animal mitochonadrial DNA: structure and evolution. Int. Rev. Cytol. 141: 173–216.

Wu, Q. L., W. Liu, B. C. Shi, Y. Gu, and S. J. Wei. 2013. The complete mitochondrial genome of the summer fruit tortrix moth *Adoxophyes orana* (Lepidoptera: Tortricidae). Mitochondr. DNA 24: 214–216.

Wu, Y. P., J. L. Zhao, T. J. Su, Q. S. He, J. L. Xie, and C. D. Zhu. 2016. The complete mitochondrial genome of *Carposina sasakii* (Lepidoptera: Carposinidae). Mitochondr. DNA 27: 1432–1434.

Wu, Y. P., J. L. Zhao, T. J. Su, J. Li, F. Yu, D. Chesters, R. J. Fan, M. C. Chen, C. S. Wu, and C. D. Zhu. 2012. The complete mitochondrial genome of *Leucoptera malifoliella* Costa (Lepidoptera: Lyonetiidae). DNA Cell. Biol. 31: 1508–1522.

Xia, J., J. Hu, G. P. Zhu, C. D. Zhu, and J. S. Hao. 2011. Sequencing and analysis of the complete mitochonadrial genome of Calinaga davids Oberthr (Lepidoptera: Nymphalidae). Acta Entomologica Sinica 54: 555–565.

Yi, J., H. Wei, T. Xin, S. Que, Z. Zou, and B. Xia. 2016a. Complete mitochondrial genome sequence of *Napialus hunanensis* (Lepidoptera: Hepialidae), the host insect of *Cordyceps hawkesii*. Mitochondr. DNA 27: 773–774.

Yi, J. Q., S. Q. Que, T. R. Xue, B. Xia, and Z. W. Zou. 2016b. Complete mitochondrial genome of Thitarodes pui (Lepidoptera: Hepialidae). Mitochondr. DNA 27: 109–110.

Zhang, D. X., J. M. Szymura, and G. M. Hewitt. 1995. Evolution and structural conservation of the control region of insect mitochondrial DNA. J. Mol. Evol. 40: 382–391.

Zhao, J. L., Y. Y. Zhang, A. R. Luo, G. F. Jiang, S. L. Cameron, and C. D. Zhu. 2011. The complete mitochondrial genome of *Spilonota lechriaspis* Meyrick (Lepidoptera: Tortricidae). Mol. Biol. Rep. 38: 3757–3764.

Zhao, J. L., Y. P. Wu, T. J. Su, G. F. Jiang, C. S. Wu, and C. D. Zhu. 2014. The complete mitochondrial genome of Acleris fimbriana (Lepidoptera: Tortricidae). Mitochondr. DNA Published online 12 December 2014. DOI:10.3109/19401736.2014.982625.

Zhu, B. J., Q. N. Liu, L. S. Dai, L. Wang, Y. Sun, K. Z. Lin, G. Q. Wei, and C. L. Liu. 2013. Characterization of the complete mitochonadrial genome of *Diaphania pyloalis* (Lepidoptera: Pyralididae). Gene 527: 283–291.

Zhu, J. Y., S. J. Wei, Q. W. Li, S. Yang, and Y. H. Li. 2012. Mitochondrial genome of the pine tip moth *Rhyacionia leptotubula* (Lepidoptera: Tortricidae). Mitochondr. DNA 23: 376–378.