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ORIGINAL ARTICLE

Complete mitochondrial genome of *Palpita hypohomalia* (Lepidoptera: Pyraloidea: Crambidae) and its phylogenetic implications

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Key words. Lepidoptera, Pyraloidea, Crambidae, *Palpita hypohomalia*, mitochondrial genome, phylogenetic position

Abstract. The complete mitochondrial genome of a pyraloid species, *Palpita hypohomalia*, was sequenced and analyzed. This mitochondrial genome is circular, 15,280 bp long, and includes 37 typical metazoan mitochondrial genes (13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes) and an A+T-rich region. Nucleotide composition is highly biased toward A+T nucleotides (81.6%). All 13 protein-coding genes (PCGs) initiate with the canonical start codon ATN, except for *cox1* which is CGA. The typical stop codon TAA occurs in most PCGs, while *nad2* and *cox2* show TAG and an incomplete termination codon T, respectively. All tRNAs have a typical clover-leaf structure, except for *trnS1* (AGN) which lacks the dihydrouridine (DHU) arm. Comparative mitochondrial genome analysis showed that the motif "ATGATAA" between *atp8* and *atp6*, and the motif "ATACTAA" between *trnS2* and *nad1* were commonly present in lepidopteran mitogenomes. Furthermore, the "ATAG" and subsequent poly-T structure, and the A-rich 3' end were conserved in the A+T-rich regions of lepidopteran mitogenomes. Phylogenetic analyses based on our dataset of 37 mitochondrial genes yielded identical topology for the Pyraloidea, and is generally identical with that recovered by a previous study based on multiple nuclear genes. In a previous study of the Crambidae, the Evergestinae was synonymized with Glaphyriinae; the present study is the first to clarify their close relationship with mitogenome data.

INTRODUCTION

Mitochondrial genes have been extensively employed in phylogenetic investigations since they are characterized by cellular abundance, absence of introns, rapid evolution and a lack of extensive recombination (Curole & Kocher, 1999; Cameron, 2014). In recent years, increasing numbers of mitochondrial genes, and even the whole mitochondrial genome (mitogenome), have become accessible following the development of sequencing technology, which in parallel has provided effective data for studies on systematics, population genetics and evolutionary biology (Cameron, 2014). A typical animal mitogenome is circular and includes 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and an A+T-rich region (Boore, 1999). Mitogenome data has been used to investigate the phylogenetic relationships of multiple animal groups, and great progression has been made (e.g. Jex et al., 2008; Cameron, 2014; Li et al., 2017). The Lepidoptera, with more than 157,000 species recorded worldwide (van Nieukerken et al., 2011), has been intensely investigated (Timmermans et al., 2014; Yang et al., 2015; Wu et al., 2016).

The Pyraloidea, with approximately 15,500 described species worldwide (van Nieukerken et al., 2011), is one of the largest superfamilies in Lepidoptera. Although the phylogeny of this superfamily has already been extensively investigated based on morphological and genetic data (e.g. Kuznetzov & Stekolnikov, 1979; Yoshiyasu, 1985; Solis & Maes, 2002; Solis, 2007; Regier et al., 2012), taxonomic and phylogenetic ambiguities still exist. For instance, the limits and definitions of the subfamilies Spilomelinae, Acentropinae and Chrysauginae in the family Crambidae still need further confirmation (Regier et al., 2012). The most speciose subfamily of pyraloid moths, Spilomelinae, was recognized as evolutionarily polyphyletic (Solis & Maes, 2002; Fan, 2013). Given the high level of phylogenetic information provided by the animal mitogenome, sequencing mitogenomes representing more pyraloid taxa would contribute to mitogenome-based phylogenetic investigation on this superfamily and related groups.

In this study, the complete mitogenome of an additional pyraloid species, *Palpita hypohomalia* Inoue, 1996, was sequenced and analyzed. *P. hypohomalia* belongs to the Spilomelinae of Crambidae and is restricted to South China. We then compared the intergenic spacer sequence

and structure of the A+T-rich region to that of other Lepidoptera representatives. Finally, phylogenetic analyses were performed based on nearly all available mitogenomes of pyraloid species. This study provides a reference for future comparative mitogenome analyses and mitogenome-based phylogenetic investigation of Pyraloidea and related groups.

MATERIALS AND METHODS

Sample collection and DNA extraction

Adult specimens of *P. hypohomalia* were sampled by light traps in Zhoukou, Henan, China. Fresh samples were initially preserved in 100% ethanol and then stored at –80°C until required for genomic DNA extraction. Specimens were identified following the morphological description by Li (2012). Total genomic DNA was extracted from the thorax muscle tissues using a DNeasy tissue kit (Qiagen, Hilden, Germany). Molecular identification was performed by blasting the newly amplified barcode sequence (see below) in GenBank, and a 100% similarity to that of *P. hypohomalia* provided by L.H. Fan et al. (China Jiliang University, Hangzhou, unpubl.) was found. Voucher specimens are deposited in the Biology Laboratory of Zhoukou Normal University, China.

Mitogenome sequencing

Complete mitogenome sequences of *P. hypohomalia* were obtained by next-generation sequencing. Briefly, the extracted whole genome DNA was quantified and fragmented to an average size of 400 bases using a Covaris M220 system with the Whole Genome Shotgun method (Covaris, Woburn, MA, USA). Then, a library was constructed using a TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA). Finally, an Illumina HiSeq 2500 was used for sequencing with the strategy of 250 paired-ends.

Mitogenome annotation and analysis

A total of 2,298,160 raw paired reads were retrieved for *P. hypohomalia*. The complete mitogenome sequence was annotated using the MITOS webserver with invertebrate genetic code (Bernt et al., 2013). tRNAScan-SE server version 1.21 (Lowe & Eddy, 1997) was used to re-identify the 22 tRNAs as well as to re-confirm their secondary structures. MEGA version 6.06 (Tamura et al., 2013) was used to re-confirm the gene boundaries by aligning the new mitogenome with other sequenced pyraloid mitogenomes.

Nucleotide sequences of the 13 PCGs were translated with both Primer Premier version 5.00 (Premier Biosoft International, Palo Alto, CA, USA) and MEGA version 6.06 (Tamura et al., 2013) to ensure the correct reading frame. Base composition and relative synonymous codon usage (RSCU) were calculated using MEGA version 6.06 (Tamura et al., 2013). Strand asymmetry was calculated according to the formulas: AT-skew = [A–T]/[A+T] and GC-skew = [G–C]/[G+C] (Perna & Kocher, 1995). Tandem repeat elements in the A+T-rich region were identified using the Tandem Repeats Finder program (http://tandem.bu.edu/trf/trf.html) (Benson, 1999).

Phylogenetic analyses

To investigate the phylogenetic implications of the *P. hypohomalia* mitogenome in Pyraloidea phylogeny, a total of 47 taxa (Table S1), namely 37 pyraloid species with sequenced mitogenomes and ten outgroup species from Lepidoptera superfamilies Geometroidea, Bombycoidea and Noctuoidea, were sampled for phylogenetic analyses. The homologous PCG sequences of all representatives were aligned by means of codon-based multiple alignments using the MAFFT algorithm within the TranslatorX online platform (Abascal et al., 2010; Katoh et al., 2017). Two rRNA and 22 tRNA genes were aligned with the Q-INS-i algorithm within the MAFFT online platform (Katoh et al., 2017). Bayesian inference (BI) and maximum likelihood (ML) methods were used to construct phylogenetic trees based on the dataset consisting of all sites of 13 PCGs, two rRNAs and 22 tRNAs.

For BI analysis, the best schemes of partition and substitution models were determined by PartitionFinder version 1.1.1 (Lanfear et al., 2012); results are shown in Table S2. Bayesian interference analysis was performed using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Two independent Markov chain Monte Carlo (MCMC) runs were performed for 8,000,000 generations sampling per 100 generations. Convergence between the two runs was established by Tracer version 1.6 (effective sample sizes > 200) (Rambaut et al., 2014). After the first 25% of yielded trees were discarded as burn-in, a 50% majority-rule consensus tree with posterior probability was generated from the remaining trees. For ML analysis, the raxmlGUI version 1.539 interface (Silvestro & Michalak, 2012) of RAxML version 7.2.6 (Stamatakis, 2006) was employed under the GTRGAMMAI model given that most sites of the dataset show GTR+I+G as the best substitution model (Table S2), and the node reliability was assessed using the ML+rapid bootstrap algorithm with 1000 replicates.

RESULTS AND DISCUSSION

General features of the *P. hypohomalia* mitogenome

The mitogenome of *P. hypohomalia* (GenBank accession number: MH013483) is double-stranded circular with 37 typical mitochondrial genes (13 PCGs, 22 tRNAs and two rRNAs) and an A+T-rich region (Fig. 1, Table 1). The assembled mitogenome is 15,280 bp in size, comparable to other completely sequenced pyraloid mitogenomes which range from 15,110 bp in *Maruca testulalis* (Zou et al., 2016) to 15,490 in *Diatraea saccharalis* (Li et al., 2011). Among the typical 37 genes, 23 are encoded on the majority strand (J-strand), and the remaining 14 genes are located on the minority strand (N-strand). This pattern is identical to that of all other sequenced Lepidoptera mitogenomes as well as other insects (e.g. Clary & Wolstenholme, 1985; Jiang et al., 2016; Wu et al., 2016; Yang et al., 2018).

In the *P. hypohomalia* mitogenome, 49 overlapping sites were detected across 11 gene junctions 1–17 bp in length. Among them, a 7-bp motif "ATGATAA" between *atp8* and *atp6* was recognized, a character commonly found in Lepidoptera (Fig. 2A) as well as other insects such as *Reduvius tenebrosus* (Hemiptera) (Jiang et al., 2016) and *Taeniopteryx ugola* (Plecoptera) (Chen & Du, 2017). In addition to the A+T-rich region, a total of 217 intergenic nucleotides across 17 gene junctions 1–46 bp in length were identified in the *P. hypohomalia* mitogenome. It has been reported that the spacer sequence between *trnS2* and *nad1* is important in mitochondrion transcription (Taanman, 1999) and could be commonly found in insect mitogenome (Wu et al., 2016). In this spacer sequence, a conserved motif "ATACTAA" could be recognized in *P. hypohomalia* as well as other reported pyraloid mitogenomes (Yang et al., 2018). Moreover, comparative mitogenome analysis showed that this motif was also commonly present in lepidopteran insects, including *Thitarodes renzhiensis*, a non-

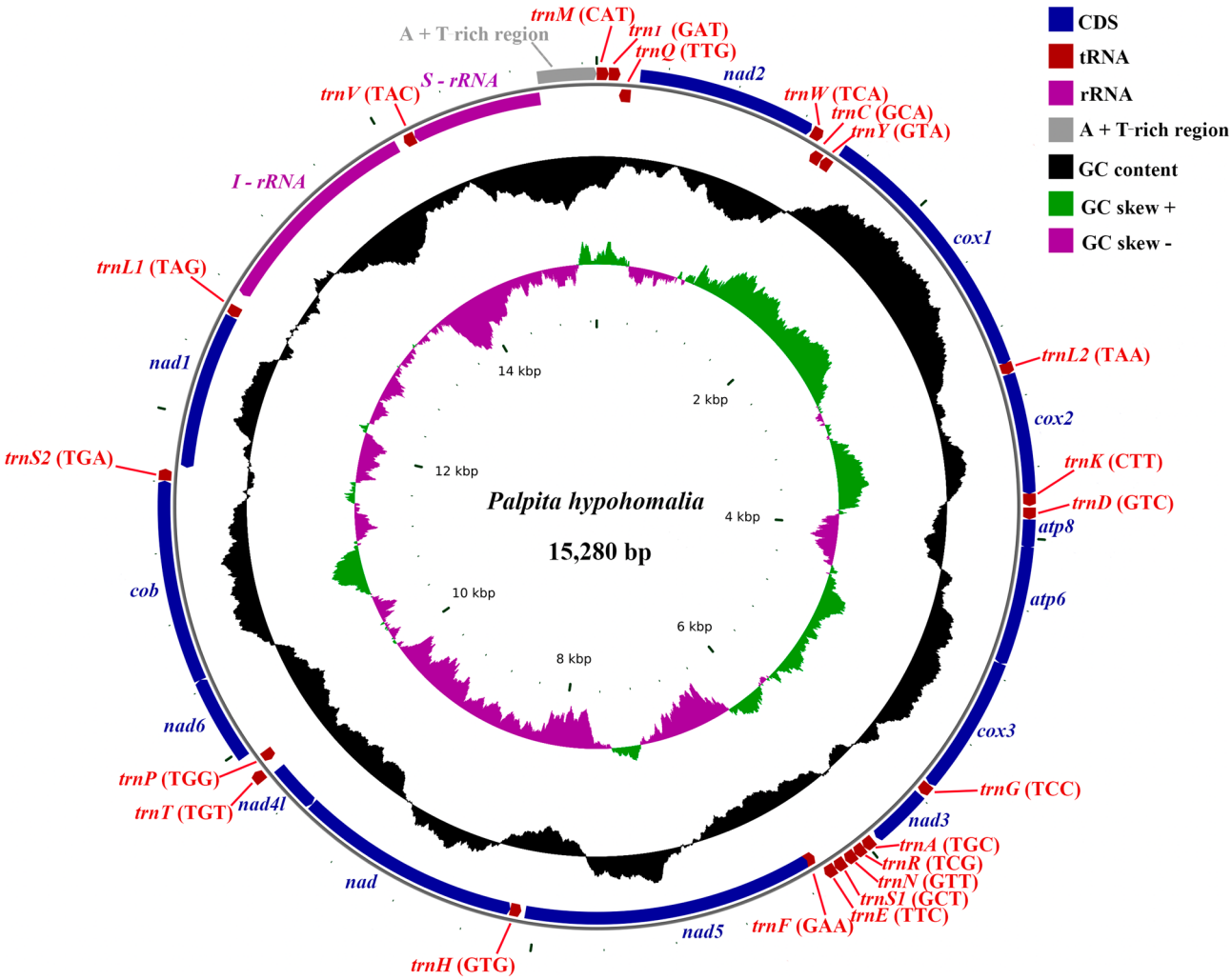

Fig. 1. Mitochondrial genome map of *Palpita hypohomalia*.

A

	atp8	*atp6*
Palpita hypohomalia (Pyraloidea)	. . . TAATAATTTAATTTGAAAATGATAAATAATCTTTTTTCAATTTTT. . .
Parnassius cephalus (Papilionoidea)	. . . TAAAAACATAAATTGAAAATGATAAGTAATTTATTTTCTATTTTC. . .
Bombyx mori (Bombycoidea)	. . . ATTAACTTTTAATTGAAAATGATAACAAACTTATTTTCTATTTTT. . .
Prays oleae (Yponomeutoidea)	. . . AAAAATATTAAACTGAAAATGATAACAAATTTATTTTCAACTTTT. . .
Rhodopsona rubiginosa (Zygaenoidea)	. . . TTATTATCTTAATTGAAAATGATAAATAATTTATTTTCTATTTTT. . .
Grapholita dimorpha (Tortricoidae)	. . . AAAAAACTTTAACTGAAAATGATAAATAATTTATTTTCAATTTTT. . .
Apocheima cinerarium (Geometroidea)	. . . AAATAATTTAACTTGAAAATGATAAGTAACTTATTTTCAATTTTC. . .
Spodoptera litura (Noctuoidea)	. . . AAAAAATTTTAATTGAAAATGATAAGTAATTTATTTTCAATTTTT. . .
Thitarodes renzhiensis (Hepialoidea)	. . . TGCTAATTTAACCTGAAAATGATAACAAATTTATTTTCAGTATTC. . .

B

	trnS2	spacer sequence	*nad1*
Palpita hypohomalia (Pyraloidea)	. . . TATTAATTT	ATACTAAAAAAATATC	TTATAAAA. . .
Parnassius cephalus (Papilionoidea)	. . . TATTAATTT	ATACTAAAATTATTAA	CTAATTTA. . .
Bombyx mori (Bombycoidea)	. . . TATTAATTT	TTTATTAATCATAAAAATATTACAA	TTAAAATA. . .
Prays oleae (Yponomeutoidea)	. . . TATTAATTT	ATACTAAAATAAATTTCTAA	TTAAAAAA. . .
Rhodopsona rubiginosa (Zygaenoidea)	. . . TATTAATTT	GTACTATAAATTTTTTAATAA	TTAAAATA. . .
Grapholita dimorpha (Tortricoidae)	. . . TATTAATTT	ATACTAAAAAAATATATAT	TTAAATAA. . .
Apocheima cinerarium (Geometroidea)	. . . TATTAATTT	ATACTAAAAAATTATAATTAT	TTATAATA. . .
Spodoptera litura (Noctuoidea)	. . . TATTAATTT	ATACTAAAAAAATTAAA	TTATAATA. . .
Thitarodes renzhiensis (Hepialoidea)	. . . TATTAATTTTATACTATTTTATAA		TTAATATA. . .

Fig. 2. A: The overlapping region between *atp8* and *atp6* in representatives of lepidopteran mitogenomes. Nucleotides colored red indicate the sequence of overlapping region, nucleotides with green underline indicate partial sequence of the *atp8* gene, and nucleotides with blue underline indicate partial sequence of the *atp6* gene. B: Spacer sequence between *trnS2* and *nad1* representatives of lepidopteran mitogenomes. Nucleotides colored red indicate the "motif" sequence, nucleotides with green underline indicate partial sequence of the *trnS2* gene, and nucleotides with blue underline indicate partial sequence of the *nad1* gene.

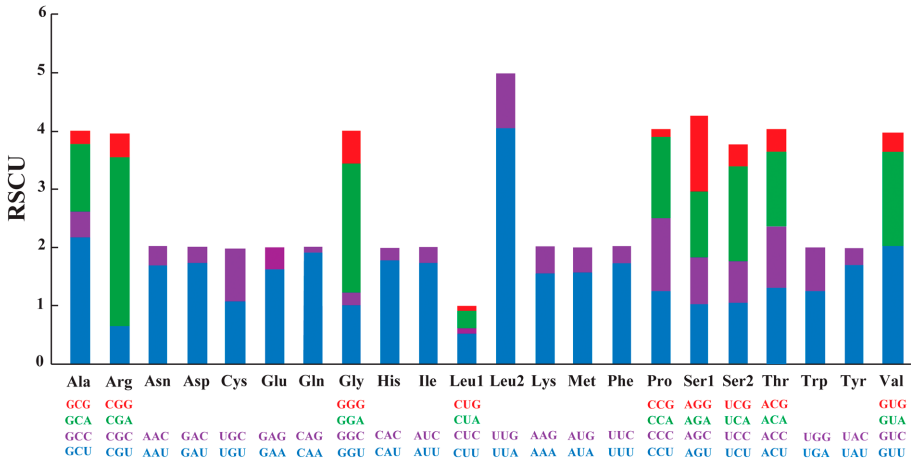

Fig. 3. Relative synonymous codon usage (RSCU) in PCGs of the *Palpita hypohomalia* mitogenome. Codon families are indicated below the X-axis.

ditrysian species of Lepidoptera, although with a change in the last base ("ATACTAT") (Fig. 2B). This phenomenon is in accordance with that reported by Cameron & Whiting (2008). Surprisingly, we did not detect this motif in *Bombyx mori* (GenBank accession number: KM875545), a representative of the Bombycoidea herein. However, in another strain of *B. mori* (GenBank accession number: AY048187), the same motif "ATACTAA" is identified. Therefore, whether the absence of this motif in *B. mori* (GenBank accession number: KM875545) can be ascribed to sequencing errors requires further investigation.

Table 1. Summary of the *Palpita hypohomalia* mitogenome.

Feature	Strand	Location	Size (bp)	Start codon	Stop codon	Anti-codon	Intergenic nucleotides
trnM	J	1–68	68			CAT	0
trnI	J	69–134	66			GAT	–3
trnQ	N	132–200	69			TTG	46
nad2	J	247–1260	1014	ATT	TAG		7
trnW	J	1268–1334	67			TCA	–8
trnC	N	1327–1395	69			GCA	3
trnY	N	1399–1464	66			GTA	3
cox1	J	1468–3003	1536	CGA	TAA		–5
trnL2 (UUR)	J	2999–3065	67			TAA	0
cox2	J	3066–3750	685	ATG	T		–3
trnK	J	3748–3818	71			CTT	8
trnD	J	3827–3893	67			GTC	0
atp8	J	3894–4055	162	ATC	TAA		–7
atp6	J	4049–4723	675	ATG	TAA		–1
cox3	J	4723–5511	789	ATG	TAA		3
trnG	J	5515–5581	67			TCC	9
nad3	J	5591–5935	345	ATT	TAA		21
trnA	J	5957–6023	67			TGC	0
trnR	J	6024–6086	63			TCG	–1
trnN	J	6086–6151	66			GTT	5
trnS1 (AGN)	J	6157–6222	66			GCT	0
trnE	J	6223–6289	67			TTC	–2
trnF	N	6288–6353	66			GAA	–17
nad5	N	6337–8088	1752	ATT	TAA		0
trnH	N	8089–8155	67			GTG	–1
nad4	N	8155–9495	1341	ATG	TAA		2
nad4l	N	9498–9785	288	ATG	TAA		2
trnT	J	9788–9854	67			TGT	0
trnP	N	9855–9919	65			TGG	38
nad6	J	9958–10458	501	ATT	TAA		–1
cob	J	10458–11606	1149	ATG	TAA		1
trnS2 (UCN)	J	11608–11673	66			TGA	16
nad1	N	11690–12625	936	ATG	TAA		1
trnL1 (CUN)	N	12627–12693	67			TAG	26
rrnL	N	12720–14071	1350				26
trnV	N	14098–14167	70			TAC	0
rrnS	N	14168–14945	778				0
A+T-rich region		14946–15280	335				0

Note: "J" indicates the majority strand and "N" indicates the minority strand in the strand column.

Nucleotide composition

In the *P. hypohomalia* mitogenome, the nucleotide composition is A = 40.5%, G = 7.6%, C = 11.4% and T = 40.5% (Table 2). The high A+T content of 81% is comparable to that of other completely sequenced pyraloid mitogenomes, which ranges from 77.0% in *Scirpophaga incertulas* (Cao et al., 2014) to 82.1% in *Chilo auricilius* (Cao & Du, 2014). Specifically, the A+T content of PCGs, tRNAs, rRNAs, and the A+T-rich region is 79.5%, 81.7%, 85.1%, and 93.2%, respectively. In addition to the A+T content, AT-skew and GC-skew were also routinely used to describe the base composition of mitochondrial genomes (Perna & Kocher, 1995; Wei et al., 2010). In the newly sequenced mitogenome, the AT-skew is zero, whereas the GC-skew is negative (Table 2). The negligible AT-skew and moderate GC-skew in the mitogenome are similar to other lepidopteran insects (Cameron & Whiting, 2008).

Protein-coding genes

The total length of the 13 PCGs is 11,173 bp, accounting for approximately 73% of the whole mitogenome (Tables 1–2). All PCGs start with the conventional ATN codon (ATT for *nad2*, *nad3*, *nad5*, and *nad6*; ATG for *cox2*, *atp6*, *cox3*, *nad4*, *nad4l*, *cob*, and *nadl*; ATC for *atp8*). The exception is *cox1*, which starts with CGA, although non-canonical start codons for *cox1* are common across insects (Fenn et al., 2007; Wu et al., 2016; Yang et al., 2018). All 13 PGCs end with the typical stop codon TAA, except for *nad2* which has TAG and *cox2* which has an incomplete termination codon T. Incomplete termination codons are commonly recognized across arthropod mitogenomes, which may be related to post-transcriptional modification during the mRNA maturation process (Ojala et al., 1981).

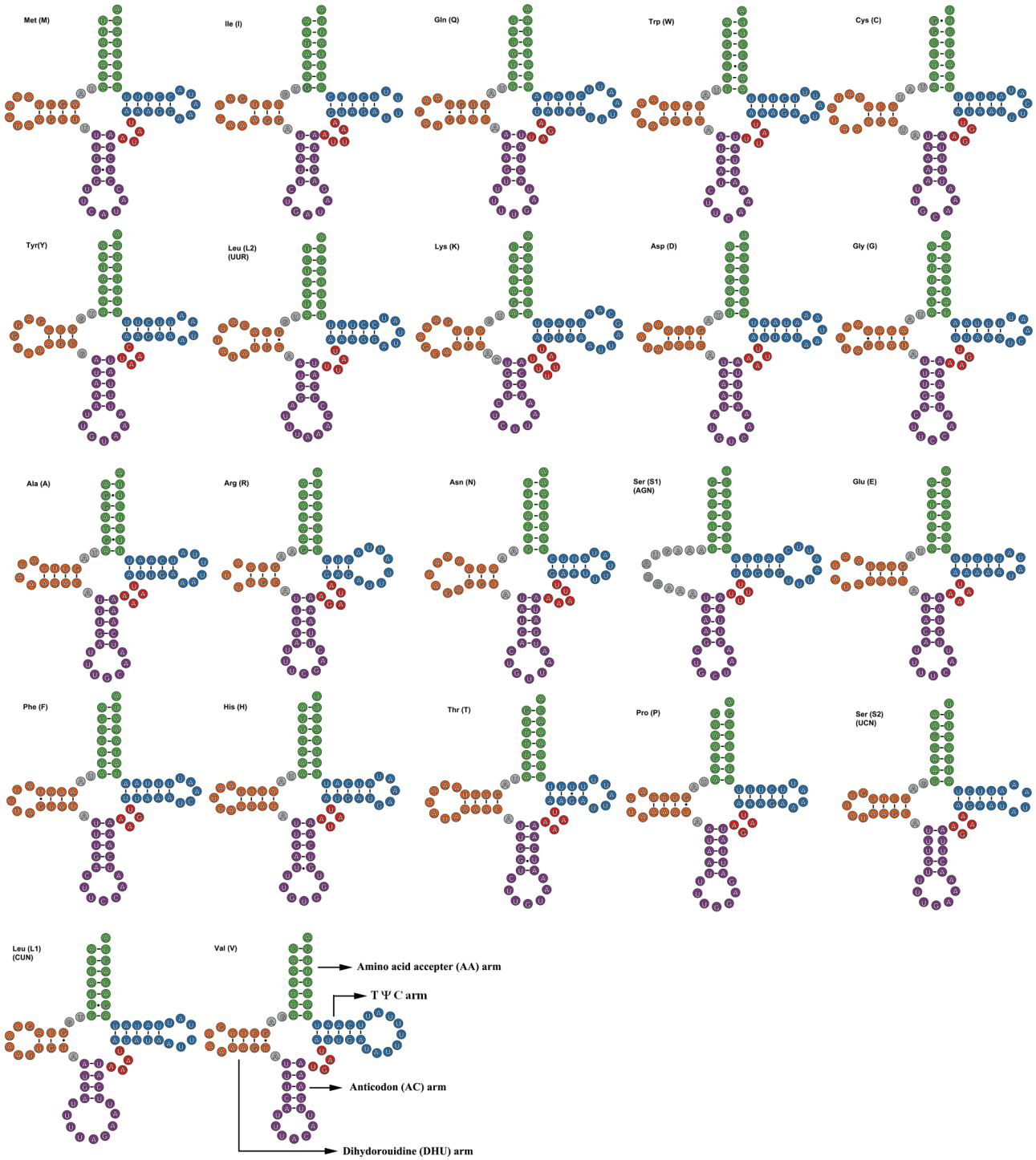

Fig. 4. Putative secondary structures of tRNAs from the *Palpita hypohomalia* mitogenome. tRNAs are labelled with the abbreviations of their corresponding amino acids. tRNA arms are illustrated as for *trnV*. Dashes indicate Watson-Crick base pairs, dots indicate wobble GU pairs, and other non-canonical pairs are not marked.

The relative synonymous codon usage (RSCU) values of the *P. hypohomalia* mitogenome are shown in Fig. 3 and Table S3. There are 3,712 codons excluding the termination codons. Among them, UUA (Leu), AUU (Ile), UUU (Phe), AUA (Met), and AAU (Asn) represent the five most frequently occurring codons (1,758/3,712; 47%). Interestingly, the codon UCG is absent in the *P. hypohomalia* mitogenome. Further comparative analysis showed that frequently occurring codons and those with higher RSCU values for each amino acid have a higher A+T content, which obviously contributes to the A+T bias of the whole mitogenome.

tRNAs and rRNAs

The *P. hypohomalia* mitogenome contains 22 typical tRNAs with lengths ranging from 63 bp (*trnR*) to 71 bp (*trnK*) (Table 1). The total length of tRNAs is 1,407 bp, accounting for approximately 9% of the whole mito-

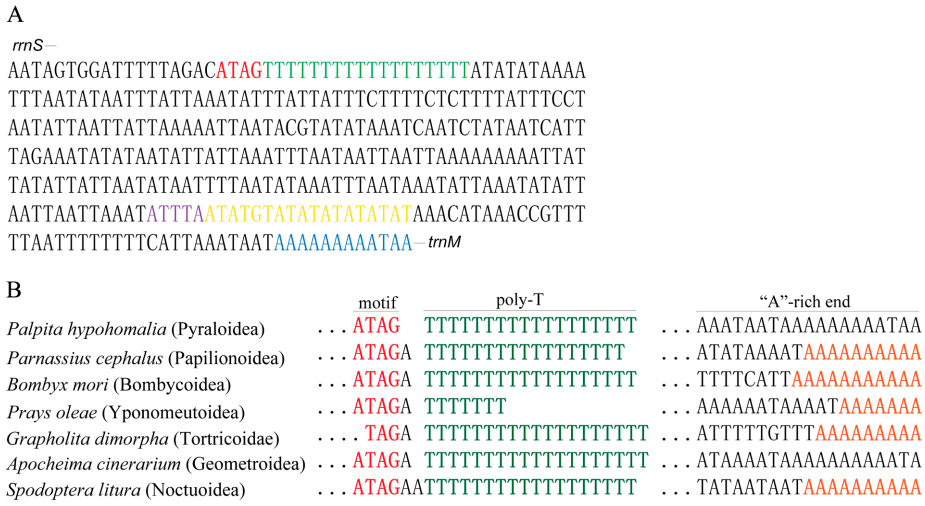

Fig. 5. A: Organization of the A+T-rich region in the *Palpita hypohomalia* mitogenome. The motif ATAG (marked red) and subsequent poly-T stretch (marked green), the motif ATTTA (marked purple) and subsequent microsatellite-like AT repeat sequences (marked yellow), and the 3' end sequences highly biased "A" bases (marked blue), are emphasized. B: Alignment of the A+T-rich regions from representatives of lepidopteran mitogenomes. The conserved motif ATAG (marked red) and subsequent poly-T stretch (marked green), and the 3' end sequences highly biased "A" bases (marked orange), are emphasized. Dots indicate omitted sequences and the number of dots is not proportional to nucleotide number of corresponding part.

genome (Table 2). Among them, eight tRNAs are encoded by N-strand and the remaining 14 by J-strand. As shown in Fig. 4, all tRNAs exhibit typical clover-leaf secondary structure with the exception of *trnS1* (AGN) which lacks the DHU arm; this feature is common to all lepidopteran insects, except in the *Adoxophyes honmai* (Tortricidae) mitogenome where all tRNAs show a complete clover-leaf structure (Lee et al., 2006). Moreover, the lack of the DHU arm in *trnS1* (AGN) is a common feature in metazoan mitogenomes (Garey & Wolstenholme, 1989; Lavrov et al., 2000). In all tRNAs of the *P. hypohomalia* mitogenome, we recognized 22 unmatched base pairs. Among them, 18 were non-canonical G-U pairs, and the remaining four were mismatched base pairs including three U-U and one G-G pairs. The overrepresented pattern of the non-canonical G-U pairs in tRNAs of the mitogenome is commonly present in other insects (Salvato et al., 2008; Chen et al., 2016; Chen & Du, 2017).

Two rRNA genes, *rrnS* and *rrnL*, were recognized in the *P. hypohomalia* mitogenome (Fig. 1, Table 1). *rrnS* is 778 bp long, located between *trnV* and the A+T-rich region; *rrnL* is 1,350 bp long, located between *trnV* and *trnL1*. The lengths of *rrnS* and *rrnL* in the *P. hypohomalia* mitogenome are comparable to that of other completely sequenced pyraloid mitogenomes, namely 765 bp in *Maruca testulalis* (Zou et al., 2016) to 791 bp in *Tyspanodes hypsalis* (Wang et al., 2016) for *rrnS*, and 1,253 bp in *Maruca testulalis* (Zou et al., 2016) to 1,442 bp in *Chilo suppressalis* (Yin et al., 2011) for *rrnL*.

Table 2. Nucleotide composition of the *Palpita hypohomalia* mitogenome.

Region	Size (bp)	A%	G%	C%	T%	A+T%	AT-skew	GC-skew
Mitogenome	15,280	40.5	7.6	11.4	40.5	81	0	–0.2
PCGs	11,173	40	8.3	12.2	39.5	79.5	0.006	–0.190
tRNAs	1,407	41.2	8	10.3	40.5	81.7	0.009	–0.126
rRNAs	2,130	43.7	9.9	5	41.4	85.1	0.027	0.329
A+T-rich region	335	44.8	2.7	4.2	48.4	93.2	–0.039	–0.217

A+T-rich region

The A+T-rich region of the *P. hypohomalia* mitogenome is located between the *rrnS* and *trnM* genes and is 335 bp in size (Fig. 1, Table 1). This length is comparable to that of other completely sequenced pyraloid mitogenomes, which ranges from 278 bp in *Meroptera pravella* (Ali et al., 2017) to 596 bp in *Plodia interpunctella* (GenBank accession number: KT207942) (Tang et al., 2015). As in other reported pyraloid mitogenomes (Yang et al., 2018), several conserved sequence blocks can be recognized in this region. These blocks include (from 5' to 3' end) the motif "ATAG" and subsequent poly-T structure, the motif "ATTTA" and subsequent macrosatellite $(AT)_n$ element, and an "A"-rich 3' end upstream of the *trnM* gene (Fig. 5A). It has been reported that these conserved blocks play a key role in replication and transcription of the mitogenome (Zhang & Hewitt, 1997). The motif "ATAG" and subsequent poly-T structure, and the nucleotide A-rich 3' end upstream of the *trnM* gene in the A+T-rich region, are common to all lepidopteran mitogenomes (Fig. 5B). The insect A+T-rich region is generally characterized by the presence of multiple tandem repeat elements (Vila & Björklund, 2004). This character has been recognized in several species such as *Chilo auricilius* and *Scirpophaga incertulas* (Cao & Du, 2014; Cao et al., 2014). However, in *P. hypohomalia* and some other reported pyraloid mitogenomes (Liu et al., 2016; Ma et al., 2016; Yang et al., 2018), no tandem repeat elements were identified.

Phylogenetic analyses

In the present study, we reconstructed the Pyraloidea phylogeny based on a dataset including 37 genes (13 PCGs, two rRNA and 22 tRNAs) of the whole mitogenome. ML and BI analyses generated identical topology as summa-

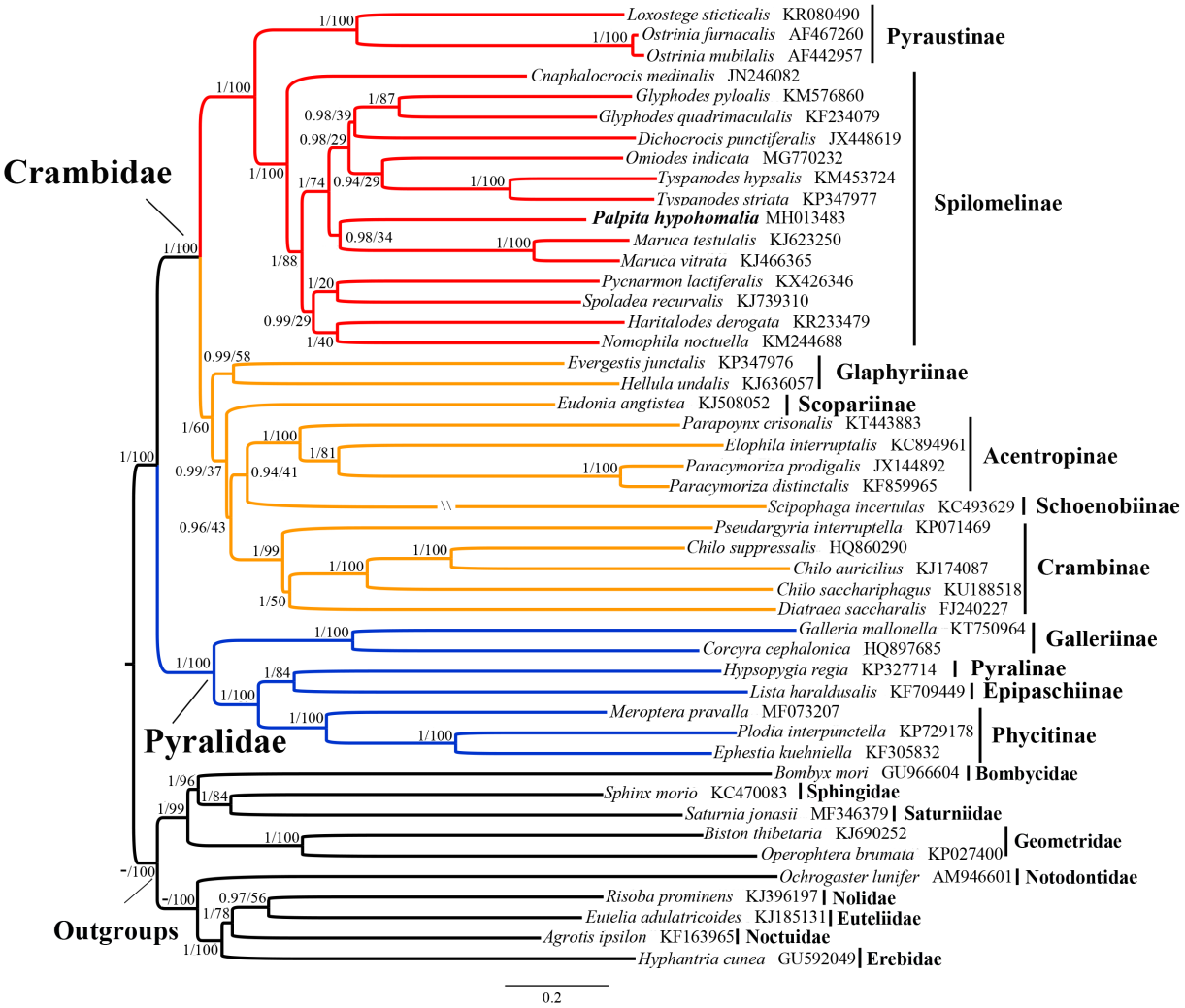

Fig. 6. Bipartition tree obtained from ML analysis based on the dataset consisting of all sites of 13 PCGs, two rRNAs and 22 tRNAs. The species with newly sequenced mitogenomes are emphasized in bold. Numbers separated by a slash on the node are the posterior probability for the BI tree and bootstrap value; the dash (–) represents an unrecovered node in BI analysis.

rized in Fig. 6. Relative to multiple outgroup species, the Pyraloidea formed a well-supported monophyly with two clades corresponding to the two pyraloid families Pyralidae and Crambidae. According to Regier et al. (2012), the Pyralidae includes five subfamilies, four of which were included in the analysis (Chrysauginae was excluded since it did not have a reported mitogenome). The relationships between them were consistently recovered as Galleriinae + (Phycitinae + (Pyralinae + Epipaschiinae)), confirming the findings of Regier et al. (2012) based on multiple nuclear genes, as well as that of Yang et al. (2018) based on the combined 13 PCGs and two rRNA genes of the mitogenome. Regarding Crambidae, we sampled all seven available mitogenomes of the ten subfamilies defined by Regier et al. (2012). Two groups in this family were consistently recovered, generally corresponding to the "*PS clade*" and "*non-PS clade*" defined by Regier et al. (2012). The well-supported sister groups Pyraustinae and Spilomelinae constituted the "*PS clade*" in this study, and the other five subfamilies (Glaphyriinae, Schoenobiinae, Scopariinae, Crambinae, and Acentropinae) formed the "*non-PS clade*". The division of two groups in Crambidae is also supported by other investigations based on 13 PCGs or a combination of 13 PCGs and two rRNAs (Ma et al., 2016; Yang et al., 2018). However, in the relationships among the five subfamilies of the "*non-PS clade*", variations exist between the present and previous studies. Our ML and BI analyses consistently recovered them as Glaphyriinae + (Scopariinae + (Crambinae + (Schoenobiinae + Acentropinae))), which shows minor difference with that of Regier et al. (2012). In Regier et al. (2012), the sister group Scopariinae + Crambinae was recovered. However, great differences in topology and node supports can be found between the results herein and that of Ma et al. (2016) and Yang et al. (2018) which were based on partial mitogenome sequences. As noted in Cameron (2014), almost all mitogenome-based phylogenetic investigations on insects have included the 13 PCGs, but the inclusion of the two rRNAs and 22 tRNAs has been more variable. Our results show that a dataset including 37 mitochondrial genes can provide more resolved pyraloid relationships than those which use 13 PCGs or a combination of 13 PCGs and two rRNAs, as in Ma et al. (2016) and Yang et al. (2018), respectively. This indicates that more phylogenetic information, possi-

bly in dataset including nuclear and the whole mitogenome sequences (Cameron, 2014), may gain better improvement on phylogeny of related insect groups. *Evergestis junctalis*, historically belonging to the Evergestinae, was transferred to the Glaphyriinae due to the synonymy between Evergestinae and Glaphyriinae suggested by Regier et al. (2012). Our results with mitogenome data allowed us to clarify the taxonomical identity of *E. junctalis* and confirm the previous work of Reiger et al. (2012).

The mitogenome sequenced in this study placed *P. hypohomalia* within the Spilomelinae of the Crambidae, which is taxonomically in accordance with morphological study (Li, 2012). The Spilomelinae, with 3,775 species assigned to 318 genera, represents the most speciose subfamily in the Pyraloidea. This large group is currently hypothesized to be polyphyletic or paraphyletic with respect to the Pyraustinae (Solis & Maes, 2002; Regier et al., 2012). However, to date, no phylogenetic investigation has been conducted to support this hypothesis or provide a robust genus-level relationship in Spilomelinae. In this study, 11 spilomeline genera with mitogenome data available were sampled and formed a well-supported group outside the Pyraustinae; the relationships were consistently recovered as *Cnaphalocrocis* + (((*Pycnarmon* + *Spoladea*) + (*Haritalodes* + *Nomophila*)) + (((*Glyphodes* + *Dichocrocis*) + (*Omiodes* + *Tyspanodes*)) + (*Palpita* + *Maruca*))). This relationship is different to that of Ma et al. (2016) and Yang et al. (2018) which included seven and ten genera of this subfamily, respectively, which could be ascribed to the different dataset used.

CONCLUSIONS

The complete mitogenome of the *P. hypohomalia* was determined using the next-generation sequencing method. Comparative mitogenome analyses showed that this mitogenome is typical of Lepidoptera mitogenomes in gene content, gene order and gene orientation. The Pyraloidea phylogeny obtained herein based on a dataset including mitochondrial 37 genes was in corroboration with a previous study based on multiple nuclear genes. In the Spilomelinae, the 11 genera involved herein formed a well-supported group and consistently showed an identical topology across analyses, which could provide reference for future investigation to resolve the limits and definitions of the subfamilies of Spilomelinae.

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Table S1. List of Lepidoptera species used in phylogenetic analyses.

Superfamily	Species	Accession no.	Size (bp)
Bombycoidea	*Bombyx mori*	GU966604	15,656
	Saturnia jonasii	MF346379	15261
	Sphinx morio	KC470083	15,299
Geometroidea	*Operophtera brumata*	KP027400	15,748
	Biston thibetaria	KJ690252	15,485
Noctuoidea	*Hyphantria cunea*	GU592049	15,481
	Agrotis ipsilon	KF163965	15377
	Eutelia adulatricoides	KJ185131	15,360
	Risoba prominens	KJ396197	15,343
	Ochrogaster lunifer	AM946601	15,593
Pyraloidea	*Elophila interruptalis*	KC894961	15,351
	Parapoynx crisonalis	KT443883	15,374
	Paracymoriza distinctalis	KF859965	15,354
	Paracymoriza prodigalis	JX144892	15,326
	Chilo auricilius	KJ174087	15,367
	Chilo sacchariphagus	KU188518	15,378
	Chilo suppressalis	HQ860290	15,465
	Diatraea saccharalis	FJ240227	15,490
	Pseudargyria interruptella	KP071469	15,231
	Evergestis junctalis	KP347976	15,438
	Hellula undalis	KJ636057	14,678
	Loxostege sticticalis	KR080490	15,218
	Ostrinia furnacalis	AF467260	14,536
	Ostrinia nubilalis	AF442957	14,535
	Scirpophaga incertulas	KC493629	15,223
	Eudonia angustea	KJ508052	15,386
	Cnaphalocrocis medinalis	JN246082	15,388
	Dichocrocis punctiferalis	JX448619	15,355
	Glyphodes pyloalis	KM576860	14,960
	Glyphodes quadrimaculalis	KF234079	15,255
	Haritalodes derogata	KR233479	15,253
	Maruca testulalis	KJ623250	15,110
	Maruca vitrata	KJ466365	15,385
	Nomophila noctuella	KM244688	15,309
	Omiodes indicata	MG770232	15,367
	Palpita hypohomalia	MH013483	15,280
	Pycnarmon lactiferalis	KX426346	15,219
	Spoladea recurvalis	KJ739310	15,273
	Tyspanodes hypsalis	KM453724	15,329
	Tyspanodes striata	KP347977	15,255
	Corcyra cephalonica	HQ897685	15,273
	Galleria mellonella	KT750964	15,320
	Lista haraldusalis	KF709449	15,213
	Ephestia kuehniella	KF305832	15,295
	Meroptera pravella	MF073207	15,260
	Plodia interpunctella	KP729178	15,287
	Hypsopygia regina	KP327714	15,212

Table S2. The best scheme and substitution models used in BI analysis.

Partitions	Models	Genes
P1	GTR + I + G	a6p1, c3p1, cbp1
P2	TVM + I + G	a6p2, c1p2, c2p2, c3p2, cbp2
P3	TrN + I + G	a6p3, c1p3, c3p3
P4	GTR + I + G	a8p1, a8p2, n2p1, n3p1, n6p1
P5	GTR + G	a8p3, n2p3
P6	GTR + I + G	c1p1, c2p1
P7	TrN + I + G	c2p3, cbp3, n3p3, n6p3
P8	GTR + I + G	n1p1, n4p1, n4lp1, n5p1
P9	GTR + I + G	n1p2, n4p2, n4lp2, n5p2
P10	TIM + G	n1p3, n4lp3
P11	TVM + I + G	n2p2, n3p2, n6p2
P12	HKY + I + G	n4p3, n5p3
P13	GTR + I + G	rrnL, rrnS
P14	GTR + I + G	tRNAs

Note: c1–c3, n1–n6, a6, a8 and cb indicate the 13 PCGs; p1, p2 and p3 indicate the first, second and third codon positions of each PCG, respectively.

Table S3. Codon usage in PCGs of the *Palpita hypohomalia* mitogenome.

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU (F)	350	1.88	UCU (S)	117	2.9	UAU (Y)	182	1.88	UGU (C)	28	1.93
UUC (F)	22	0.12	UCC (S)	10	0.25	UAC (Y)	12	0.12	UGC (C)	1	0.07
UUA (L)	475	5.23	UCA (S)	74	1.83	UAA (*)	11	1.83	UGA (W)	92	1.96
UUG (L)	17	0.19	UCG (S)	0	0	UAG (*)	1	0.17	UGG (W)	2	0.04
CUU (L)	28	0.31	CCU (P)	68	2.16	CAU (H)	62	1.85	CGU (R)	14	1.06
CUC (L)	2	0.02	CCC (P)	9	0.29	CAC (H)	5	0.15	CGC (R)	1	0.08
CUA (L)	21	0.23	CCA (P)	48	1.52	CAA (Q)	60	1.94	CGA (R)	35	2.64
CUG (L)	2	0.02	CCG (P)	1	0.03	CAG (Q)	2	0.06	CGG (R)	3	0.23
AUU (I)	440	1.89	ACU (T)	85	2.34	AAU (N)	227	1.82	AGU (S)	24	0.59
AUC (I)	25	0.11	ACC (T)	6	0.17	AAC (N)	23	0.18	AGC (S)	2	0.05
AUA (M)	266	1.83	ACA (T)	53	1.46	AAA (K)	92	1.88	AGA (S)	95	2.35
AUG (M)	24	0.17	ACG (T)	1	0.03	AAG (K)	6	0.12	AGG (S)	1	0.02
GUU (V)	60	1.86	GCU (A)	81	2.55	GAU (D)	57	1.78	GGU (G)	55	1.13
GUC (V)	2	0.06	GCC (A)	5	0.16	GAC (D)	7	0.22	GGC (G)	3	0.06
GUA (V)	58	1.8	GCA (A)	39	1.23	GAA (E)	66	1.71	GGA (G)	107	2.21
GUG (V)	9	0.28	GCG (A)	2	0.06	GAG (E)	11	0.29	GGG (G)	29	0.6