

Characterization of the complete mitochondrial genome of the Oriental armyworm, *Mythimna separata* (Lepidoptera: Noctuidae)

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Abstract. The complete mitochondrial genome (mitogenome) of *Mythimna separata* (Lepidoptera: Noctuidae) was determined to be 15,329 bp, including 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes and an A+T-rich region. The AT skew of this mitogenome was slightly negative and the nucleotide composition was also biased toward A+T nucleotides (81.00%). All PCGs were initiated by ATN codons, except for cytochrome c oxidase subunit 1 (*cox1*) gene, which was initiated by CGA. Five of the 13 PCGs have the incomplete termination codon, T or TA. All the tRNA genes displayed a typical clover-leaf structure of mitochondrial tRNA. The A+T-rich region of the mitogenome was 372 bp in length and consisted of several features common to the Noctuidae. Phylogenetic analysis confirmed the placement of *M. separata* within the Noctuidae.

INTRODUCTION

The insect mitochondrial genome (mitogenome) is a circular molecule, 14–19 kb in size and contains 22 transfer RNA (tRNA) genes and two ribosomal RNA genes encoding the small and large subunit rRNAs (*rrnS* and *rrnL*) that are involved in the translation of 13 protein coding genes (PCGs), ATPase subunits 6 and 8 (*atp6* and *atp8*), cytochrome c oxidase subunits 1–3 (*cox1–cox3*), cytochrome B (*cob*), NADH dehydrogenase subunits 1–6 and 4L (*nad1–6* and *nad4L*), and a large non-coding element termed the A+T-rich region, containing both the origin for replication and transcription (Moritz et al., 1987; Wolstenholme, 1992; Cameron, 2014). Because of their unique features, including coding content conservation, maternal inheritance, and rapid evolution, mitogenome sequences have been widely used as an informative molecular marker for diverse evolutionary studies among species in the fields of molecular evolution, phylogenetics, population genetics, and comparative and evolutionary genomics (Harrison, 1989; Boore, 1999; Whinnett et al., 2005; Lopez-Vaamonde et al., 2012; Timmermans et al., 2014).

Whilst the Lepidoptera rank as the second most numerous order of insects, comprising > 160,000 species, classified into 45–48 superfamilies, to date only a few complete or near-complete lepidopteran mitogenomes are available. These include species in the superfamilies Bombycoidea, Geometroidea, Noctuoidea, Pyraloidea, Tortricidae, Hepialoidea and Papilionoidea. The superfamily Noctuoidea is a very large assemblage that accounts for about 40% of all described Lepidoptera (Speidel & Naumann, 2004);

however, only a few mitogenomes of this superfamily have so far been sequenced (Table 1).

The Oriental armyworm, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) is a seasonal migrating agricultural pest moth in China that causes serious economic losses, and as such, has been used as a test insect to explore new agricultural pesticides (Chen et al., 1995; Fan et al., 2014). A better understanding of the lepidopteran mitogenome requires an expansion of taxon and genome samplings. Characterization of the *M. separata* mitogenome has advanced knowledge of lepidopteran mitogenomes and provided new insights into understanding the mechanisms underlying mitochondrial DNA (mtDNA) evolution, especially in terms of gene rearrangements. In the present study, the complete mitogenome of *M. separata* was sequenced and the mitogenome was then subjected to phylogenetic analyses, involving comparison with selected species belonging to the orders Lepidoptera, Orthoptera and Diptera (see below).

MATERIAL AND METHODS

DNA extraction

Adult *M. separata* of both sexes were collected in Yancheng, Jiangsu Province. Total DNA from individuals was isolated using the Genomic DNA Extraction Kit (Sangon Biotech Co., Shanghai, China), according to the manufacturer's instructions. Extracted DNA was used for PCR amplification of the complete mitogenome.

PCR amplification and sequencing

For PCR amplification of the entire *M. separata* mitogenome, nine primer sets designed in accordance with the known mito-

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TABLE 1. List of the complete mitogenome of the superfamily Noctuoidea.

Family / species	Size (bp)	Accession No.	Reference
Notodontidae			
<i>Phalera flavescent</i>	15,659	JF440342	Sun et al., 2012
<i>Ochrogaster lunifer</i>	15,593	AM946601	Salvato et al., 2008
Arctiidae			
<i>Hyphantria cunea</i>	15,481	GU592049	Liao et al., 2010
<i>Amata formosae</i>	15,463	KC513737	Lu et al., 2013
Doidae			
<i>Doa</i> sp.	15,228	KJ508058	Timmermans et al., 2014
Erebidae			
<i>Nyctemera arctata albofasciata</i>	15,432	KM244681	Tang et al., 2014
Lymantriidae			
<i>Lymantria dispar</i>	15,569	FJ617240	Zhu et al., 2010
<i>Gynaephora menyanensis</i>	15,570	KC185412	Yuan & Zhang, 2013
Noctuidae			
<i>Helicoverpa armigera</i>	15,347	GU188273	Yin et al., 2010
<i>Sesamia inferens</i>	15,413	JN039362	Chai & Du, 2012
<i>Ctenoplia agnata</i>	15,261	KC414791	Gong et al., 2013
<i>Spodoptera exigua</i>	15,365	JX316220	Wu et al., 2013a
<i>Agrotis ipsilon</i>	15,377	KF163965	Wu et al., 2013b
<i>Agrotis segetum</i>	15,378	KC894725	Wu et al., 2013c
<i>Spodoptera litura</i>	15,374	KF543065	Liu et al., 2014a
<i>Helicoverpa punctigera</i>	15,382	KF977797	Walsh, 2014
<i>Noctua pronuba</i>	15,315	KJ508057	Timmermans et al., 2014
<i>Acronicta psi</i>	15,350	KJ508060	Timmermans et al., 2014
<i>Striacosta albicosta</i>	15,553	KM488268	Coates B.S. & Abel C.A., unpubl.
<i>Mythimna separata</i>	15,329	KM099034	This study

chondrial sequences from other noctuids, were synthesized by Sunbiotech (Co., Ltd. Beijing, China) (Table 2). Mitochondrial DNA sequences were amplified using Aidlab 2×PCR Master Mix (Beijing, China) according to the manufacturer's instructions. PCR was performed under the following conditions: a 4 min denaturation step at 94°C, followed by 35 cycles of 30 s at 94°C, a 1–3 min annealing step at 50–60°C, concluding with a 10 min extension step at 72°C. The products were separated by agarose gel electrophoresis (1% w/v) and purified using a DNA Gel Extraction Kit (Transgen Co., Beijing, China). The purified products were then ligated into the T-vector (SangonBiotech Co., Shanghai, China) and sequenced at least three times.

Sequence assembly and gene annotation

Sequence annotation was performed using the blast tools available at NCBI web site (<http://blast.ncbi.nlm.nih.gov/Blast>) and using DNASTar package (DNASTar Inc. Madison, USA). Identification of tRNA genes was verified using the tRNAscan-SE program. The potential stem-loop secondary structures within these tRNA gene sequences was calculated using the tRNAscan-SE Search Server (<http://lowelab.ucsc.edu/tRNAscan-SE/>) (Lowe & Eddy, 1997). The secondary structures of tRNA genes that could not be predicted using the tRNAscan-SE were analyzed by comparison with the nucleotide sequences of other insect tRNA sequences (Yin et al., 2010; Chai & Du, 2012; Gong et al., 2013; Wu et al., 2013a). The PCGs were identified by sequence similarity with that of the beet armyworm, *Spodoptera exigua* (Hübner) (Noctuidae) (Wu et al., 2013a). The nucleotide sequences of PCGs were translated with the invertebrate mitogenome genetic code. Alignments of PCGs from various lepidopteran mitogenomes was performed using Clustal X (Thompson et al., 1997), whilst composition skewness was calculated according to the formulas: AT skew = $[A - T] / [A + T]$; GC skew = $[G - C] / [G + C]$ (Junqueira et al., 2004). Tandem repeats in the A+T-rich region

were predicted using the Tandem Repeats Finder program (<http://tandem.bu.edu/trf/trf.html>) (Benson, 1999).

Phylogenetic analysis

To reconstruct the phylogenetic relationship among lepidopteran insects, the complete mitogenomes of lepidopteran species were obtained from the GenBank database. These mitogenomes were divided into seven lepidopteran suborders, including, as aforementioned, Bombycoidea, Geometroidea, Hepialoidea, Noctuoidea, Pyraloidea, Tortricoidea and Papilionoidea. The mitogenomes of *Locusta migratoria* L. (NC_001712), *Drosophila yakuba* Burla (NC_001322) and *Anopheles gambiae* Giles (NC_002084) were used as outgroups. The amino acid sequences of each of the 13 mitochondrial PCGs were aligned using default settings and concatenated. The concatenated set of amino acid sequences from the 13 PCGs was used in phylogenetic analysis, which were performed using the Maximum Likelihood method using the MEGA version 6.06 program. This method was used to infer phylogenetic trees with 1000 bootstrap replicates. Substitution model selection was also conducted based on the lowest BIC scores (Bayesian Information Criterion) using MEGA 6.06. The mtREV24 + G + F model was the appropriate models for the amino acid sequence dataset (Tamura et al., 2013).

RESULTS AND DISCUSSION

Genome organization and base composition

The complete mitogenome of *M. separata* is a closed circular molecule of size 15,329 bp. The gene content is typical of other insect mitochondrial genomes, including 22 tRNA genes (one for each amino acid, two for leucine and serine), 13 PCGs (*cox1–3*, *nad1–6*, *nad4L*, *cob*, *atp6* and *atp8*), two mitochondrial ribosomal RNAs (*rrnS* and *rrnL*), and a major non-coding region known as the A+T-

TABLE 2. Primers used for amplification of the mitogenome of *Mythimna separata*.

Primer pair	Primer sequence (5' → 3')
F1	GCTTTTGGGCTCATACCTCA
R1	GATGAAATACCTGCAAGATGAAG
F2	TGGAGCAGGAACAGGATGAAC
R2	GAGACCACTACTTGCTTTCAG
F3	ATTTGTGGAGCTAATCATAG
R3	GGTCAGGGACTATAATCtAC
F4	TCGACCTGGAACCTTAGC
R4	GCAGCTATAGCCGCTCCTACT
F5	TAAAGCAGAAACAGGAGTAG
R5	ATTGCGATATTATTCTTTTG
F6	ACATTCTTAGGTGGATTA
R6	GTAAAGTGGCATTATCT
F7	GGAGCTTCTACATGAGCTTTTGG
R7	GTTTGCACCTCGATGTTG
F8	GGTCCCTTACGAATTTGAATATATCCT
R8	AAACTAGGATTAGATACCCTATTAT
F9	CTCTACTTTGTTACGACTTATT
R9	TCTAGGCCAATTCAACAACC

rich region (Fig. 1) (Table 3). The order of genes and the orientation of the mitogenome of *M. separata* are identical to the Noctuid mitogenomes. Compared with the ancestral insect gene order, the placement of the *trnM* tRNA gene in the *M. separata* mitogenome differs from that of ancestral insects. In *M. separata*, the order is as follows: A+T-rich region, *trnM*, *trnI*, *trnQ*, *nad2*, whereas in the ancestral order it is: A+T-rich region, *trnI*, *trnQ*, *trnM*, *nad2* (Fig. 2) (Boore et al., 1998). The placement of *trnM* may therefore represent a molecular feature exclusive to lepidopteran mtDNAs (Lavrov et al., 1999). The tRNA gene rearrangements are commonly considered to be a consequence of tandem duplication of part of the mitogenome, followed by random (or nonrandom) loss of duplicated copies (Lavrov et al., 2002; Carapelli et al., 2006; Juhling et al., 2012). However, the ancestral arrangement of the *trnM* gene cluster was also found in the ghost moths; their ancestral gene order indicates that this gene rearrangement event likely occurred after Hepialoidea diverged from other lepidopteran lineages (Cao et al., 2012).

Overlapping and intergenic spacer regions

The mitogenome of *M. separata* has a total of 31 bp overlap between genes in seven locations, the longest 8 bp overlap being located in two locations: *trnW* and *trnC* (Table 3). The overlapping between *trnW* and *trnC* is also detected in other lepidopteran species, such as *Pieris* (*Artogeia*) *melete* Ménétries (Hong et al., 2009), *Antheraea pernyi* Guérin-Méneville (Liu et al., 2008), *Ostrinia furnacalis* (Guenée) and *Ostrinia nubilalis* (Hübner) (Coates et al., 2005). A seven nucleotide overlap between *atp8* and *atp6* is common to many other insect mitogenomes, such as *Samia cynthia cynthia* (Drury) (Sima et al., 2013), *Eriogyna pyretorum* (Westwood) (Jiang et al., 2009), *Chilo suppressalis* (Walker) (Yin et al., 2011), *Phthonandria atrilineata* (Butler) (Yang et al., 2008), *Actias selene* (Hüb-

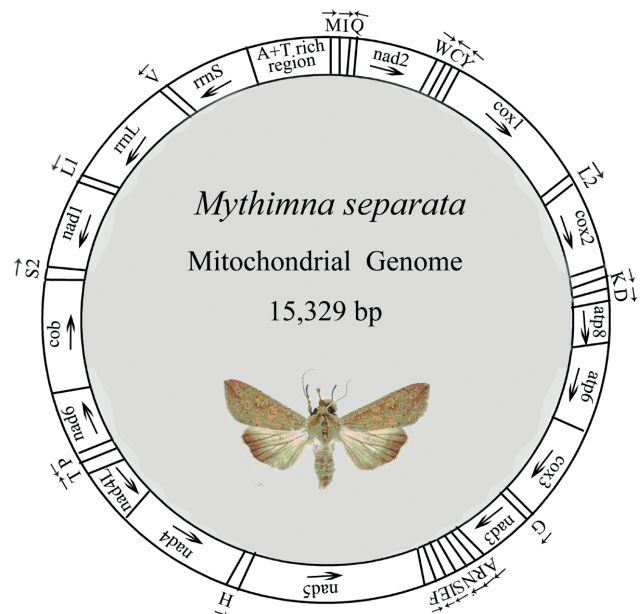


Fig. 1. Map of the mitogenome of *Mythimna separata*. tRNA genes are labelled according to the IUPAC-IUB. Single letter amino acids above the bar indicate the coding sequence on the major strand, below the coding on the minor strand. Anti-clockwise PCGs or rRNA genes are located on the L strand, whilst other genes are located on the H strand.

ner) (Liu et al., 2012a), *Diaphania pyloalis* Walker (Zhu et al., 2013), and *Bombyx mori* L., strains H9 and Dazao (Dai et al., 2013; Liu et al., 2013). The mitogenome of *M. separata* contains 162 bp of intergenic spacer sequence spread over 16 regions, ranging in size from 1 to 50 bp (Table 3), which is considerably longer than that of other Lepidoptera species, including *Manduca sexta* (L.) (115 bp over 13 regions), *A. selene* (137 bp over 13 regions), but shorter than *Coreana raphaelis* (Oberthür). (178 bp over 17 regions), *D. pyloalis* (194 bp over 16 regions), and *Ochrogaster lunifer* Herrich-Schäffer (371 bp over 20 regions). The longest spacer sequence is 50 bp, located between the *trnQ* and *nad2* genes, and is extremely A+T rich.

Skewness

The nucleotide composition of the mitogenome of *M. separata* was found to be as follows: A = 6135 (40%), T = 6282 (41%), G = 1175 (7.7%) and C = 1737 (11.3%). As observed in other lepidopterans, the nucleotide composition of the *M. separata* mitogenome is biased toward A+T (81.00%) (Table 4) which was higher than other lepidopteran species such as *O. lunifer* (77.84%), *A. selene* (78.91%), *Amata formosae* Butler (79.49%), *Ctenophusia agnata* (Staudinger) and *Lymantria dispar* (L.) (79.90%),

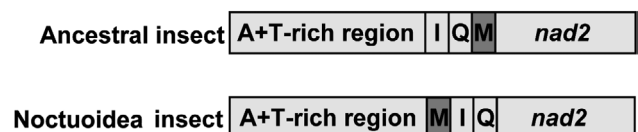


Fig. 2. The *trnM* gene rearrangement in the *M. separata* comparison with an ancestral insect.

TABLE 3. Summary of the mitogenome of *Mythimna separata*.

Gene	Direction	Location	Size	Anticodon	Start codon	Stop codon	Intergenic nucleotides
<i>trnM</i>	F	1–68	68	CAT	—	—	0
<i>trnI</i>	F	69–133	65	GAT	—	—	–3
<i>trnQ</i>	R	131–199	69	TTG	—	—	50
<i>nad2</i>	F	250–1258	1009	—	ATT	T	0
<i>trnW</i>	F	1259–1328	70	TCA	—	—	–8
<i>trnC</i>	R	1321–1385	65	GCA	—	—	5
<i>trnY</i>	R	1391–1455	65	GTA	—	—	2
<i>cox1</i>	F	1458–2994	1537	—	CGA	T	–3
<i>trnL2(UUR)</i>	F	2992–3058	67	TAA	—	—	0
<i>cox2</i>	F	3059–3740	682	—	ATT	T	0
<i>trnK</i>	F	3741–3811	71	CTT	—	—	20
<i>trnD</i>	F	3832–3898	67	GTC	—	—	0
<i>atp8</i>	F	3899–4057	159	—	ATT	TAA	–7
<i>atp6</i>	F	4051–4727	677	—	ATG	TA	0
<i>cox3</i>	F	4728–5516	789	—	ATG	TAA	2
<i>trnG</i>	F	5519–5583	65	TCC	—	—	0
<i>nad3</i>	F	5584–5937	354	—	ATT	TAA	9
<i>trnA</i>	F	5947–6013	67	TGC	—	—	–1
<i>trnR</i>	F	6013–6077	65	TCG	—	—	5
<i>trnN</i>	F	6083–6149	67	GTT	—	—	–3
<i>trnS1(AGN)</i>	F	6147–6217	71	GCT	—	—	1
<i>trnE</i>	F	6219–6285	67	TTC	—	—	8
<i>trnF</i>	R	6294–6360	65	GAA	—	—	4
<i>nad5</i>	R	6365–8110	1746	—	ATT	TAA	0
<i>trnH</i>	R	8111–8176	66	GTG	—	—	0
<i>nad4</i>	R	8177–9515	1339	—	ATG	T	24
<i>nad4L</i>	R	9540–9830	291	—	ATG	TAA	2
<i>trnT</i>	F	9833–9897	65	TGT	—	—	0
<i>trnP</i>	R	9898–9962	65	TGG	—	—	7
<i>nad6</i>	F	9970–10,503	534	—	ATC	TAA	2
<i>cob</i>	F	10,506–11,654	1149	—	ATG	TAA	3
<i>trnS2(UCN)</i>	F	11,658–11,724	65	TGA	—	—	18
<i>nad1</i>	R	11,743–12,687	945	—	ATA	TAA	–6
<i>trnL1(CUN)</i>	R	12,682–12,749	68	TAG	—	—	0
<i>rrnL</i>	R	12,750–14,108	1359	—	—	—	0
<i>trnV</i>	R	14,109–14,174	66	TAC	—	—	0
<i>rrnS</i>	R	14,175–14,957	783	—	—	—	0
A+T-rich region		14,958–15,329	372	—	—	—	—

A. pernyi (80.15%), *Sesamia inferens* (Walker) (80.24%), *Antheraea yamamai* Guérin-Méneville (80.29%), *Hyphantria cunea* (Drury) and *Adoxophyes honmai* Yasuda (80.39%), *Caligula boisduvalii* (Eversmann) (80.62%), *Agrotis segetum* Schiff (80.70%), *E. pyretorum* (80.82%), and *S. exigua* (80.93%); however, it was slightly lower in the case of *P. atrilineata* (81.02%), *Spodoptera litura* (F.) (81.03%), *C. agnata* (81.10%), *Agrotis ipsilon* Hufnagel, (81.25%), *Gynaephora menyanensis* Yan & Chou (81.48%), *B. mori C108* (81.36%), Japanese *B. mandarina* (Moore) (81.68%), *M. sexta* (81.79%) and *C. raphaelis* (82.66%). The AT skew and GC skew were calculated for the selected complete lepidopteran mitogenomes (Table 4). The AT skew for the *M. separata* mitogenome was slightly negative (–0.012), indicating a higher occurrence of T over A nucleotides. Similar results were observed

in *A. formosae* (–0.027), *Phalera. flavescentis* (Bremer & Gery) (–0.009), *C. agnata* (–0.024), *A. ipsilon* (–0.006), *A. segetum* (–0.004), *C. raphaelis* (–0.047), *C. boisduvalii* (–0.024), *A. selene* (–0.023), *A. yamamai* (–0.022), *A. pernyi* (–0.021), *M. sexta* (–0.005) and *A. honmai* and *S. inferens* (–0.001). The AT skew of tRNA and rRNA genes in the *M. separata* mitogenome was 0.004 and –0.021, respectively. The AT skew in the *M. separata* mitogenome A+T-rich region was –0.054, indicating a bias for T over A nucleotides. The GC skew values were negative in all sequenced lepidopteran mitogenomes, indicating a higher content of C compared to G nucleotides. In *M. separata*, the GC skew for rRNA genes was –0.382, which was lower than that observed for tRNA, PCGs and the A+T-rich region, indicating a heavy bias toward C versus G nucleotides in genes encoding rRNAs.

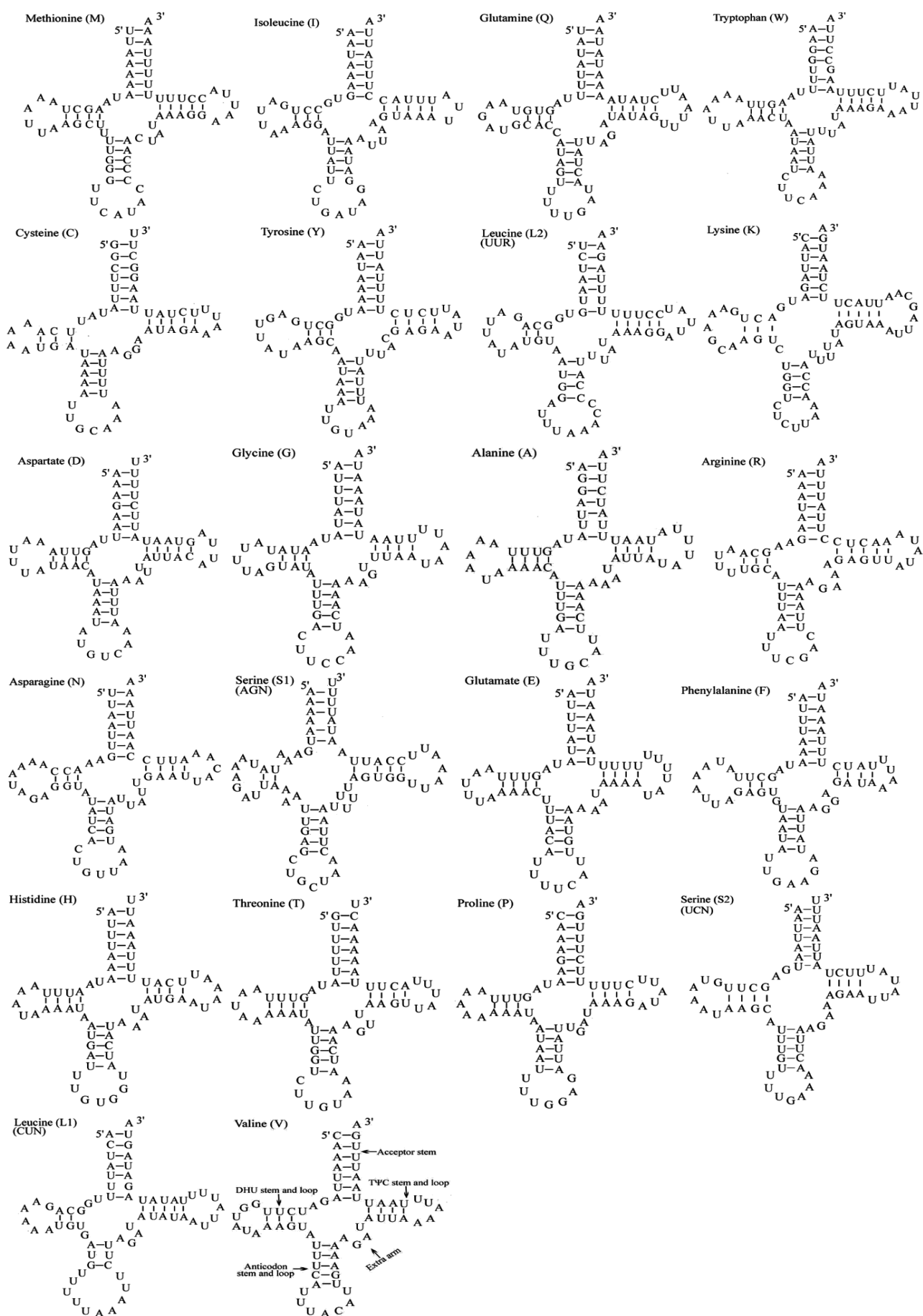


Fig. 3. Putative secondary structures for the tRNA genes of the *Mythimna separata* mitogenome.

rrnS-14,958 ATATGTAAATTTTAAAGATAGAAATTTTTTTTTT
TTTTTATATTAAAAATTTAATAAATTATTAATTTTAAATA
ATTTCTTTTCTTTCTTTTATAAATTAATATTAACCTAAAT
TGGAAATTAACAATTAATACTATAAATTATAATATTAAT
ATAATTAATTAATTTTTTAAATAATTTATATATATATATATA
TTAATATATTAATTTTAAATTTATTAATAAATATTAATATTA
AATGTTTAATATGTATATATATATATATATATTAAT
TCTTTTAAAAAATTTTAAACCATTCTTAATAATTTACATATA
AATAAAAAAATAATA 15,329 - *trnM*

Fig. 4. Features present in the A+T-rich region of *Mythimna separata*. The sequence is shown in the reverse strand. The coloured nucleotides sequentially indicate the ATATG motif (orange), poly-T stretch (blue), two microsatellite T/A repeat sequences (red), and a poly-A stretch (green). 51 bp of two tandem repeats are shown underlined in red and black.

Protein-coding genes

All protein-coding sequences in the *M. separata* mitogenome were initiated by typical ATN codons (*nad1* gene for ATA, *nad6* gene for ATC, *cob*, *atp6*, *cox3*, *nad4* and *nad4L* genes for ATG, *atp8*, *cox2*, *nad2*, *nad3*, and *nad5* genes for ATT), with the exception of the *cytochrome oxidase subunit 1* (*cox1*) (Table 3). The sequence of the 13 PCGs is 11,211 bp in length, whilst the arrangement of the PCGs is same as that in the other sequenced lepidopterans. The A+T nucleotide composition of 13 PCGs in the mitogenome of *M. separata* was 79.6%. Sequence alignment revealed that the open reading frame of *cox1* in *M. separata* also starts with a CGA codon for encoding arginine. Different from the other PCGs, *cox1* is initiated by a CGA codon, this non-canonical putative start codon also being found in some other insects (Yin et al., 2010; Chai & Du, 2012; Gong et al., 2013). The TTAG tetra-nucleotide is a putative start codon in *C. raphaelis* (Kim et al., 2006), whilst the sequence TATTAG also represents a putative start codon in the moth species, *O. nubilalis* and *O. furnicalis* (Coates et al., 2005). An unusual start codon for the *cox1* gene has also been described in various arthropod mtDNAs (Negrisolo et al., 2004). The canonical termination codon (TAA) occurs in 8 PCGs in the *M. separata* mitogenome. The remaining five PCGs, including *atp6*, *cox1*, *cox2*, *nad2*, and *nad4*, were terminated with a single T or TA. The *cox2* gene has incomplete stop codons, as found in all lepidopteran mtDNA sequenced to date.

Transfer RNA genes

The structure of tRNA genes was predicted using the tRNAscan-SE Search Server. The *M. separata* mitogenome contains 22 tRNA genes, similar to the mitogenomes of most animals (Boore, 1999). A total of 15 unmatched base pairs were found to occur in the *M. separata* mitochondrial tRNA genes; ten of them are G-U pairs, which form a weak bond. The *trnL1*, *L2*, *trnV* and *trnA* genes contain an U-U mismatch. All tRNAs displayed the typical clover-leaf secondary structure observed in mitochondrial tRNA genes (Fig. 3); *trnS1* (AGN) lacking a stable dihydrouridine (DHU) arm has been observed in several insects and metazoan mitogenomes (Wolstenholme, 1992; Sima et al., 2013; Liu et al., 2014b). Among the 22 tRNA genes,

14 were encoded by the H-strand, eight by the L-strand (Table 3). The A+T content of all tRNAs was 81.8%.

Ribosomal RNA genes

As typically observed in other insect mitogenomes, two rRNA genes (*rrnS* and *rrnL*) were present in the *M. separata* mitogenome, which were located between *trnL1* (CUN) and *trnV*, and between *trnV* and the A+T-rich region, respectively. The lengths of the *rrnL* and *rrnS* were 1359 bp and 783 bp, respectively. The A+T content of the two rRNA genes as analyzed were 84.73%, which is within the range reported for other lepidopterans (Table 4).

The A+T-rich region

The A+T-rich region of *M. separata* extends over 372 bp (14,958–15,329 nt) and is located between the *rrnS* and *trnM* genes. Comparison with other lepidopteran species revealed that this region is shorter than that of *C. boisduvalii* (330 bp), *A. yamamai* (334 bp), *C. raphaelis* (375 bp), *P. atrilineata* (457 bp), *A. melete* (351 bp), *A. honmai* (489 bp), *B. mori* (494 bp), *A. pernyi* (552 bp) and *B. mandarina* (747 bp), and longer than that of *M. sexta* (324 bp) and *O. lunifer* (319 bp). The A+T-rich region contained the highest A+T content (94.35%) in the mitogenome, which is within the range reported for other lepidopteran insects (Table 4). Several conserved structures of other lepidopteran mitogenomes were also observed in the A+T-rich region of the *M. separata* mitogenome, including the motif “ATAGA” and a 20-bp poly-T stretch at the downstream of the *rrnS* gene, which is widely conserved in lepidopteran mitogenomes (Timmermans et al., 2014), and may represent the origin of minority or light strand replication (Taanman, 1999). A poly-A commonly observed in other lepidopteran mitogenomes was also found immediately upstream of the *trnM* gene. We also identified microsatellite (TA)₆ and (TA)₈ elements in the A+T-rich region of the *M. separata* mitogenome. The presence of multiple tandem repeat elements is a characteristic of the insect A+T-rich region; however, the repeat element is variable, even within the same species (Pan et al., 2008; Hu et al., 2010). The *A. melete* and *Artogeia napi* L. mitogenomes contain a duplicate 36 bp repeat element comprising a 26 bp core sequence flanked by 10 bp perfectly inverted repeats (Hong et al., 2009). In *A. pernyi*, the A+T-rich region harbours a repeat element of 38 bp, occurring six times in tandem, while in the wild type of *A. pernyi*, this element is present in five repeats (Liu et al., 2012b). In *M. separata*, a tandem repeat was also found in the A+T-rich region of the mitogenome containing a duplicate 51 bp repeat element and occurring twice (Fig. 4).

Phylogenetic analyses

According to the most recent consensus view of lepidopteran relationships, Bombycoidea, Noctuoidea and Geometroidea are designated as the Macrolepidoptera, and Pyraloidea with Macrolepidoptera as Obtectomera. In the present study, the concatenated amino acid sequences of the 13 PCGs of the mitochondrial genome were used to reconstruct the phylogenetic relationships among the seven superfamilies of Lepidoptera (Fig. 5). Phylogenetic

TABLE 4. Composition and skewness in Noctuoidea mitogenomes.

Species	Size (bp)	A%	G%	T%	C%	A+T%	AT skewness	GC skewness
<i>M. separata</i>	15,329	40.02	7.67	40.98	11.33	81.00	-0.012	-0.193
<i>P. flavesceus</i>	15,659	40.07	7.87	40.80	11.26	80.87	-0.009	-0.177
<i>O. lunifer</i>	15,593	40.09	7.56	37.75	14.60	77.84	0.030	-0.318
<i>H. cunea</i>	15,481	40.58	7.55	39.81	12.06	80.39	0.010	-0.230
<i>A. formosae</i>	15,463	38.67	7.53	40.83	12.98	79.49	-0.027	-0.266
<i>L. dispar</i>	15,569	40.58	7.57	39.30	12.55	79.88	0.016	-0.248
<i>G. menyuanensis</i>	15,770	40.88	6.77	40.60	11.75	81.48	0.003	-0.269
<i>H. armigera</i>	15,347	40.54	7.69	40.43	11.34	80.97	0.001	-0.192
<i>H. punctigera</i>	15,382	40.70	7.58	40.66	11.06	81.35	0.001	-0.187
<i>S. inferens</i>	15,413	40.06	7.61	40.17	12.15	80.24	-0.001	-0.230
<i>C. agnata</i>	15,261	39.58	7.71	41.52	11.20	81.10	-0.024	-0.185
<i>S. exigua</i>	15,365	40.87	7.68	40.06	11.39	80.93	0.010	-0.195
<i>A. ipsilon</i>	15,377	40.38	7.71	40.87	11.04	81.25	-0.006	-0.178
<i>A. segetum</i>	15,378	40.20	7.80	40.50	11.50	80.70	-0.004	-0.192
<i>A. rubrescens</i>	15,382	40.70	7.58	40.66	11.06	81.35	0.001	-0.187
<i>S. litura</i>	15,374	41.03	7.60	40.00	11.36	81.03	0.013	-0.198
<i>S. albicosta</i>	15,553	40.15	7.88	39.18	12.79	79.33	0.012	-0.238
PCGs								
<i>M. separata</i>	11,211	39.43	8.38	40.17	12.03	79.59	-0.009	-0.179
<i>P. flavesceus</i>	11,211	33.73	10.95	45.23	10.08	78.97	-0.146	0.041
<i>O. lunifer</i>	11,266	32.47	12.08	43.26	12.19	75.73	-0.142	-0.004
<i>H. cunea</i>	11,205	33.59	810.99	45.00	10.42	79.59	-0.145	0.027
<i>A. formosae</i>	11,224	33.37	11.21	44.43	10.99	77.81	-0.142	-0.010
<i>L. dispar</i>	11,236	33.22	11.26	44.62	10.90	77.84	-0.146	0.016
<i>G. menyuanensis</i>	11,228	34.63	10.18	45.15	10.04	79.78	-0.132	0.007
<i>H. armigera</i>	11,203	34.25	10.62	45.18	9.95	79.43	-0.138	0.033
<i>H. punctigera</i>	11,206	40.06	8.31	39.82	11.82	79.88	0.003	-0.174
<i>S. inferens</i>	11,232	33.54	11.08	45.09	10.29	78.62	-0.147	0.037
<i>C. agnata</i>	11,238	34.13	10.46	45.77	9.63	79.91	-0.146	0.041
<i>S. exigua</i>	11,201	34.10	10.83	45.37	9.70	79.47	-0.142	0.055
<i>A. ipsilon</i>	11,211	34.24	10.64	45.56	9.55	79.81	-0.142	0.054
<i>A. segetum</i>	11,214	33.89	10.95	45.17	10.00	79.05	-0.143	0.045
<i>A. rubrescens</i>	11,211	34.45	10.48	45.44	9.63	79.89	-0.138	0.042
<i>S. litura</i>	11,206	40.30	8.38	39.26	12.06	79.56	0.013	-0.180
<i>S. albicosta</i>	11,210	39.46	8.79	37.99	13.76	77.46	0.019	-0.220
tRNAs								
<i>M. separata</i>	1473	41.07	8.15	40.73	10.05	81.81	0.004	-0.104
<i>P. flavesceus</i>	1485	41.62	7.81	40.61	9.97	82.22	0.012	-0.121
<i>O. lunifer</i>	1666	41.78	7.32	39.86	11.04	81.63	0.023	-0.202
<i>H. cunea</i>	1474	41.86	7.87	39.89	10.38	81.75	0.024	-0.138
<i>A. formosae</i>	1467	40.49	7.98	40.22	11.32	80.71	0.003	-0.173
<i>L. dispar</i>	1469	41.66	7.96	39.35	11.03	81.01	0.029	-0.162
<i>G. menyuanensis</i>	1504	41.29	7.38	41.76	9.57	83.05	-0.006	-0.129
<i>H. armigera</i>	1473	41.41	8.15	40.39	10.05	81.81	0.012	-0.104
<i>H. punctigera</i>	1482	41.63	8.16	40.22	9.99	81.85	0.017	-0.101
<i>S. inferens</i>	1410	41.35	10.71	40.14	7.80	81.49	0.015	0.157
<i>C. agnata</i>	1477	41.23	8.19	40.22	10.36	81.45	0.012	-0.117
<i>S. exigua</i>	1475	41.56	8.07	40.07	10.31	81.63	0.018	-0.122
<i>A. ipsilon</i>	1477	41.23	8.12	40.42	10.22	81.65	0.010	-0.114
<i>A. segetum</i>	1474	40.84	8.21	40.77	10.18	81.61	0.001	-0.107
<i>A. rubrescens</i>	1483	41.67	8.16	40.19	9.98	81.86	0.018	-0.100
<i>S. litura</i>	1473	42.23	7.94	39.58	10.25	81.81	0.032	-0.127
<i>S. albicosta</i>	1486	40.58	8.48	40.44	10.50	81.02	0.002	-0.106
rRNAs								
<i>M. separata</i>	2142	41.46	4.72	43.28	10.55	84.73	-0.021	-0.382
<i>P. flavesceus</i>	2198	41.31	4.73	44.04	9.92	85.35	-0.032	-0.354
<i>O. lunifer</i>	2157	41.96	4.82	40.19	13.03	82.15	0.022	-0.460
<i>H. cunea</i>	2234	42.08	4.92	42.75	10.25	84.83	-0.008	-0.351
<i>A. formosae</i>	2163	38.93	4.72	44.85	11.51	83.77	-0.071	-0.418
<i>L. dispar</i>	2150	42.79	4.79	41.81	10.60	84.60	0.012	-0.377
<i>G. menyuanensis</i>	2311	41.89	4.28	42.84	10.99	84.73	-0.011	-0.439
<i>H. armigera</i>	2189	41.75	4.89	43.40	9.96	85.15	-0.019	-0.341
<i>H. punctigera</i>	2198	41.95	4.73	43.54	9.78	85.49	-0.019	-0.348
<i>S. inferens</i>	2169	42.60	11.20	41.49	4.70	84.09	0.013	0.409
<i>C. agnata</i>	2112	40.01	5.07	44.65	10.27	84.66	-0.055	-0.334
<i>S. exigua</i>	2196	42.35	4.64	42.81	10.20	85.15	-0.005	-0.374
<i>A. ipsilon</i>	2162	41.58	5.00	43.57	9.85	85.15	-0.023	-0.327
<i>A. segetum</i>	2164	41.54	4.85	43.62	9.98	85.17	-0.024	-0.346
<i>A. rubrescens</i>	2198	41.95	4.73	43.54	9.78	85.49	-0.019	-0.348
<i>S. litura</i>	2218	42.65	4.73	42.34	10.28	84.99	0.004	-0.370
<i>S. albicosta</i>	2147	42.06	4.84	41.92	11.18	83.98	0.002	-0.396
A+T-rich region								
<i>M. separata</i>	372	44.62	2.42	49.73	3.23	94.35	-0.054	-0.143
<i>P. flavesceus</i>	541	42.14	2.22	49.72	5.91	91.86	-0.083	-0.454
<i>O. lunifer</i>	319	44.50	1.60	48.90	5.00	93.40	-0.047	-0.524
<i>H. cunea</i>	357	45.66	1.12	49.30	3.92	94.96	-0.038	-0.556
<i>A. formosae</i>	482	42.95	2.90	49.79	4.36	92.74	-0.074	-0.201
<i>L. dispar</i>	435	45.29	1.61	50.80	2.30	96.09	-0.057	-0.176
<i>G. menyuanensis</i>	449	43.65	2.45	49.67	4.23	93.32	-0.065	-0.266
<i>H. armigera</i>	328	44.51	1.22	50.61	3.66	95.12	-0.064	-0.500
<i>H. punctigera</i>	327	45.26	1.22	51.07	2.45	96.33	-0.060	-0.335
<i>S. inferens</i>	311	43.41	1.29	52.41	2.89	95.82	-0.094	-0.383
<i>C. agnata</i>	334	46.71	1.50	46.71	5.09	93.41	0.000	-0.545
<i>S. exigua</i>	331	43.20	2.72	50.15	3.93	93.35	-0.074	-0.182
<i>A. ipsilon</i>	332	46.08	1.51	48.80	3.61	94.88	-0.029	-0.410
<i>A. segetum</i>	332	45.78	0.90	48.49	4.82	94.28	-0.029	-0.685
<i>A. rubrescens</i>	327	45.26	1.22	51.07	2.45	96.33	-0.060	-0.335
<i>S. litura</i>	326	46.63	2.15	47.24	3.99	93.87	-0.006	-0.300
<i>S. albicosta</i>	385	43.12	1.30	49.87	5.71	92.99	-0.073	-0.629

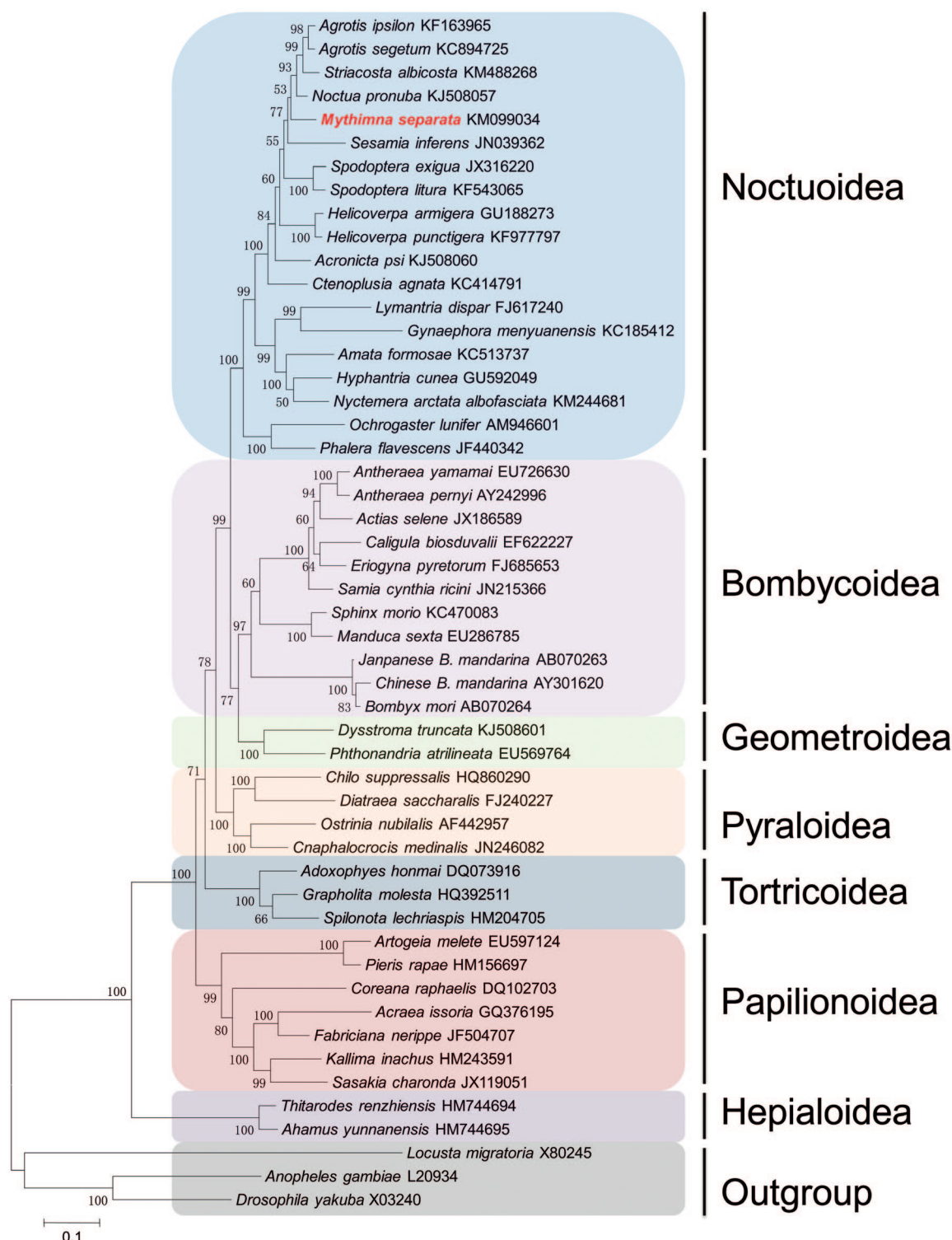


Fig. 5. Phylogeny of lepidopteran insects. Phylogenetic trees inferred from the amino acid sequences of 13 PCGs of the mitogenome using Maximum Likelihood (ML) analysis. *L. migratoria*, *D. yakuba* and *A. gambiae* were used as outgroups. The numbers above the branches specify bootstrap percentages (1000 replicates).

analyses showed that the Noctuoidea were clustered in one branch in the phylogenetic tree. Bombycoidea was the closest to Noctuoidea. More mitogenomes from lepidopteran insects are required to resolve uncertainties concerning the position of the Noctuoidea and the relationships among these superfamilies. Our phylogenetic analyses of these superfamilies supports the traditional morphology-

based classification (Kristensen & Skalski, 1999; Timmermans et al., 2014).

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