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ORIGINAL ARTICLE

Comparative analysis of the mitochondrial genome of *Hypospila bolinoides* and *Lygephila dorsigera* (Lepidoptera: Noctuoidea: Erebidae), with implications for their phylogeny

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Abstract. To analyse the phylogenetic relationships of members of the family Erebidae, the mitogenomes of two species namely, *Hypospila bolinoides* Guenée, 1852 and *Lygephila dorsigera* (Walker, 1865) were sequenced. *H. bolinoides* and *L. dorsigera* have circular genomes that are 15,640 bp and 15,567 bp long, respectively. Despite their different lengths, both genomes contain an identical gene order, which includes 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes and an A+T-rich region. The nucleotide composition of the *H. bolinoides* mitogenome vis-à-vis the A+T rich region was 80.08%, which is higher than that of *L. dorsigera* (78.03%). The AT skew and GC skew were mostly negative for both species. The A+T-rich regions of *H. bolinoides* and *L. dorsigera* were 287 bp and 383 bp long, respectively, and confined to common characteristics of Noctuoidea. Concatenated sequences of 13 PCGs from 170 taxa belonging to the five families of the superfamily Noctuoidea and two butterfly mitogenomes used as outgroups were utilized for Bayesian inference (BI) and Maximum Likelihood (ML) analyses. The first-ever comprehensive analysis of the mitogenomes of the genera *Lygephila* (Billberg, 1820) and *Hypospila* (Guenée, 1852) revealed that the superfamily Noctuoidea has a well supported monophyletic relationship with (Notodontidae + (Erebidae + (Nolidae + (Euteliidae + Noctuidae))). In support of previous partial genome analyses, the present study provides phylogenomic evidence that *L. dorsigera*, hitherto classified as a member of the subfamily Erebinae, can be placed within the subfamily Toxocampinae.

INTRODUCTION

In order to establish and infer phylogenetic relationships for diverse groups of organisms, particularly insects, the complete mitochondrial genome, also known as the mitogenome, is extensively used to address phylogenetic implications (Wolstenholme, 1992; Wilson et al., 1985; Cameron, 2014; Ma et al., 2015; Cheng et al., 2016). The insect mitochondrial genome is a relatively small genome with rapid evolutionary rates, low levels of recombination and maternal inheritance. The mitochondrial DNA (mtDNA) has a higher mutation rate and, as a result of its high copy number, large amounts of mtDNA can be assembled for genomic sequencing (Boore, 1999, 2006; Curole & Kocher, 1999; Nardi et al., 2003). These characteristic features of the mtDNA and the utilization of the complete mitogenome provide novel information concerning the classification of insects and assessments of their evolution. The insect mitochondrial genome is a double-stranded, circular molecule of 14–19 kb in length encoding 13 protein-coding genes (PCGs): two ATPase genes (*atp6* and *atp8*), three cytochrome c oxidase genes 1–3 (*cox1–cox3*), one cytochrome B (*cob*), seven NADH dehydrogenase genes (nad1-6 and nad4L), 22 transfer RNA (tRNA), two ribosomal RNA (*rrnL* and *rrnS*) genes; and a non-coding A+T-rich region (Wolstenholme, 1992; Shadel & Clayton, 1993; Boore, 1999; Jiang et al., 2009; Cameron, 2014; Sivasankaran et al., 2017; Riyaz et al., 2021, 2023; Shah et al., 2022).

The Noctuoidea comprises around 42,407 species and is the largest superfamily in the order Lepidoptera (van Nieukerken et al., 2011). Phylogenetic studies of the superfamily Noctuoidea mainly use molecular methods based on one or two genes and a few taxa (Weller et al., 1994; Mitchell et al., 1997, 2006; Fang et al., 2000). Zahiri et al. (2011) propose separating the traditional group of quadrifid noctuids from the re-established families Erebidae and Nolidae. However, this is contrary to the results of previous morphological and molecular studies. Nevertheless, their

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analysis failed to resolve the phylogenetic relationships between Erebidae subfamilies (Zahiri et al., 2012). So far, six families are recognized in the superfamily Noctuoidea: Oenosandridae, Notodontidae, Erebidae, Euteliidae, Nolidae and Noctuidae (van Nieukerken et al., 2011; Zahiri et al., 2011; Kononenko & Pinratana, 2013). With 1,760 genera and 24,569 species, Erebidae is the largest family in the superfamily Noctuoidea (van Nieukerken et al., 2011). The family Erebidae includes a great diversity of moths and, due to this and the wide range of variability, the members of this family are very difficult to describe based on their morphological features.

Hypospila is a genus of moths belonging to the family Erebidae. Guenée in 1852 erected the genus with *Hypospila bolinoides*its as the type species. Poole (1989) categorized *Hypospila* in the subfamily Ophiderinae, which Kitching & Rawlins (1999) referred to as the Calpinae in Kristensen (1999). Poole (1989) included 13 species in the genus *Hypospila*, including *Moepa* Walker, 1865 (preoccupied), *Orrea* Walker, 1866 and *Tochara* Moore, 1882 as junior synonyms of *Hypospila* Guenée, 1852. According to Holloway (1979), this genus, is made up of an allopatric variety of bolinoides-related species, such as pseudobolinoides Holloway (Solomon Is.) and similis Tams (Fiji, Rotuma, Vanuatu, Samoa). In addition, Holloway (2005) placed *Hypospila* as miscellaneous genera, while Kononenko & Pinratana (2013) placed *Hypospila* in the tribe Acantholipini of the subfamily Erebinae, which was later confirmed by the molecular phylogenetic study of Zahiri et al. (2012). Guenée, 1852 originally described *Hypospila bolinoides* as a species of moth in the Erebidae family and the type species of the genus *Hypospila*. This species is reported in the Indo-Australian tropics of China, Japan, New Guinea, Queensland and the Caroline Islands in the east, as well as India, Sri Lanka, Myanmar and the Andaman Islands (Holloway, 2005). The tiny larvae drop or leap off when alarmed and feed on young leaves of *Derris* (Leguminosae) and *Brachypterum scandens* (Fabaceae) (Robinson et al., 2010).

Lygephila is a genus of moths that belongs to the family Erebidae. Billberg established the genus in 1820, with *Phalaena lusoria* Linnaeus as its type species. Poole (1989) placed *Lygephila* in the subfamily Ophiderinae, which Kitching & Rawlins (1999) regarded as Calpinae in Kristensen (1999). Poole (1989) classified *Asticta* Hübner, 1823, *Toxocampa* Guenée, 1841, and *Eccrita* Lederer, 1857 as junior synonyms of *Lygephila* Billberg, 1820 and lists 35 species in the genus *Lygephila*. Following Beck (1960), Merzheevskaya (1988) placed *Scoliopteryx* in its own subfamily, Scoliopteryginae, and grouped *Catocala*, *Ectypa* [= *Euclidia*] and *Lygephila* together in the Catocalini. Notably, Merzheevskaya (1988) used characteristics of the setae of larvae to differentiate the present erebine taxa, *Catocala* and *Euclidia*, from *Lygephila* [Toxocampinae]. Kononenko & Fibiger (2008) placed the genus *Lygephila* in the subfamily Catocalinae, while Holloway (2005) and Kononenko & Pinratana (2013) placed it in the tribe Toxocampini under the subfamily Erebinae. In addition, the placement of the genus *Lygephila* in the subfamily Toxocampinae was later confirmed by the molecular phylogenetic study of Zahiri et al. (2012). *Lygephila dorsigera* belongs to the subfamily Toxocampinae and the family Erebidae. Francis Walker provided the first description of this species in 1865 under the name *Toxocampa dorsigera* with Sri Lanka as the species' type locality. It is found in India, Nepal, Sri Lanka, Myanmar, Thailand, China (Guangxi, Hunan, Zhejiang) and Taiwan (Holloway, 2005). Although *Lygephila cucullata* (Moore, 1882) and *Lygephila orientalis* (Butler, 1886) are frequently referred to as relatives or even synonyms of *L. dorsigera* in the literature, these two species are in different species groups within the genus *Lygephila*, which are not even remotely similar to *L. dorsigera* in terms of their external and genital structures (Fibiger et al., 2008; Pekarsky, 2013).

This is the first report of the complete mitochondrial genomes of two species of moths in the superfamily Noctuoidea, belonging to two subfamilies, Erebinae (*H. bolinoides*) and Toxocampinae (*L. dorsigera*). The newly sequenced mitogenomes were annotated, and the broad characteristics of the sequences were analysed and compared. The evolutionary relationships of the mitogenomes of 170 different lepidopteran taxa were examined and a phylogenetic tree was reconstructed using the maximum likelihood approach and Bayesian inference to assess their relationships with other Lepidoptera.

MATERIALS AND METHODS

Sample collection and genomic DNA extraction

The samples of *Hypospila bolinoides* (10.235367N, 77.492933E) and *Lygephila dorsigera* (10.23915N, 77.497716E) were collected using light traps in the Tamil Nadu part of the Western Ghats. After identification, the specimens were kept in 100 per cent ethanol and stored at –80°C until DNA isolation. The genomic DNA was extracted from tissue in the thorax of the moth according to the manufacturer's protocol using the Quick-DNA Tissue/Insect Microprep Kit (Zymo Research, Irvine, CA, USA). The quality of the DNA samples was verified using Nanodrop 1000 and validated using 1% agarose gel electrophoresis.

Mitogenome sequencing

The samples that passed the quality check were used to prepare the library. In brief, the Truseq Nano library preparation kit (Illumina) was used to prepare an indexed library based on 100 ng of DNA. Following the manufacturer's procedure, final libraries were quantified using a Qubit 4.0 fluorometer (Thermofisher) and a DNA HS assay kit (Thermofisher). The library was queried on Agilent Tapestation 4150 using extremely sensitive D1000 screen tapes (Agilent) following the manufacturer's protocol to determine the insert size. Molsys Scientific Pvt. Ltd performed the next-generation sequencing (Bangalore, India) on a NOVASEQ 6000 platform (Illumina, San Diego, CA, USA) using 150 bp paired-end reads with the sequencing depth of 4 GB per sample.

Sequence assembly and annotation

The raw sequences were assembled using NOVOPLASTY Ver. 4.2 (https://github.com/ndierckx/NOVOPlasty) (Dierckxsens et al., 2017). The sequences were annotated using MITOS2, an online web server (http://mitos2.bioinf.uni-leipzig.de/index.py) (Bernt et al., 2013). Using the genetic code for invertebrate mitogenomes through CHLOROBOX-GeSeq-Annotation of

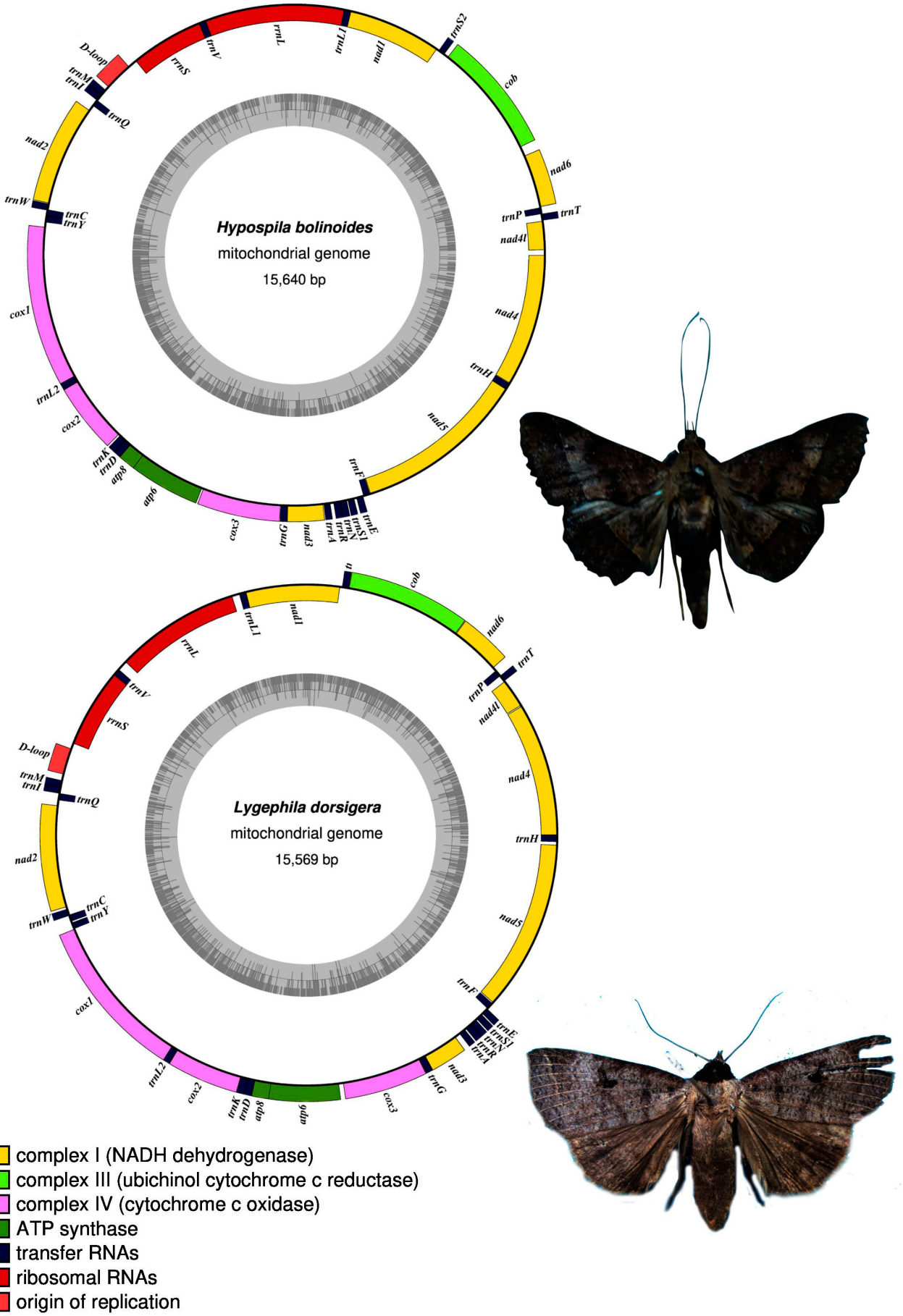

Fig. 1. Circular maps of the newly sequenced mitochondrial genomes of *H. bolinoides* and *L. dorsigera*.

Organellar Genomes (https://chlorobox.mpimp-golm.mpg.de/geseq.html), the precise lengths of 13 protein-coding genes were validated (Tillich et al., 2017). The composition skewness was calculated using the formula AT skew = [A–T]/[A+T], while GC skew = [G–C]/[G+C] (available at https://en.vectorbuilder.com/tool/gc-content-calculator). The tRNA genes and their cloverleaf structures were predicted using the MITOS2 programme and were then compared with the nucleotide sequences of other lepidopteran tRNA sequences. The putative secondary structures of tRNA genes that the MITOS2 server could not predict, were predicted using an online Bioinformatics Web Server for RNA (http://rtools.cbrc.jp/cgi-bin/index.cgi). The tandem repeats in the A+T-rich region were located using the Tandem Repeats Finder programme (http://tandem.bu.edu/trf/trf.html). Manual calculations were done to determine the overlapping areas and intergenic spacers between genes. The Relative Synonymous Codon Usage (RSCU) of PCGs was assessed using MEGA X (Kumar et al., 2018). To construct circular maps of the mitogenomes, the programme OGDRAW-Draw Organelle Genome Maps (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) (Greiner et al., 2019) was utilized (Fig. 4.4). The sequence data were tested for substitution saturation in each the 13 PCGs using applications available in DAMBE 5 (Xia, 2013).

Phylogenetic analyses

Based on the concatenated sequences of 172 species of Noctuoidea (including the two newly sequenced mitogenomes) and two butterfly mitogenomes (*Papilio polytes* and *Trogonoptera brookiana* (Papilionoidea, Papilionidae)), which were used as outgroups, phylogenetic trees were reconstructed using BI and ML methods (Table S1) (Fig. 5a–b). In addition to the two newly sequenced mitogenomes, those of 168 species of Noctuoid that belonged to five families of Noctuoidea were obtained from the GenBank: (Erebidae, Nolidae, Euteliidae, Noctuidae and Notodontidae). The analyses were done using a data set consisting of thirteen PCGs, which resulted in a similar tree topology (Fig. 4).

In order to do the phylogenetic analysis, the amino acid sequences from each of 13 PCGs genes were aligned and concatenated through codon-based multiple alignments using the MAFFT (Multiple Alignment using Fast Fourier Transform) algorithm with the FFT-NS-i strategy in the normal alignment mode (Katoh & Standley, 2013). The conserved regions were identified and poorly aligned sequences within the datasets were deleted using G-blocks (Castresana, 2000). Afterwards, the 13 PCGs were then utilized to reconstruct the phylogenetic tree, which was executed via IQ-TREE in the PhyloSuite V1.2.2 software package (https://github.com/dongzhang0725/PhyloSuiten) using Model-based Maximum Likelihood (Zhang et al., 2020). The appropriate model General Reversible Mitochondrial (mtREV) Gamma distributed with invariant sites (G+I) was utilized to infer the evolutionary connections based on 5000 bootstrap replicates. The best partitioning schemes and models for this dataset were chosen using PartitionFinder2 in PhyloSuite. Using the software package MrBayes 3.2.6 in PhyloSuite V1.2.2, the dataset was subjected to Bayesian inference (BI) using the best model (GTR+I+G), or generic time reversible model with invgamma rate variation across variable sites. The analysis indicated sufficient convergence to ensure the split frequencies' 0.01 average standard deviation. The BI analysis was done with a million generations and four chains (MCMC, three hot and one cold), sampling every 1000 generations with a burn-in of 25% of sampled values. FigTree v.1.4.4 (Rambaut, 2012) (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize and edit the phylogenetic trees.

List of abbreviations

Leu – Leucine, Phe – Phenylalanine, Ile – Isoleucine, Asn – Asparagine, Lys – Lysine, Tyr – Tyrosine, Val – Valine, Ala – Alanine, Pro – Proline, Arg – Arginine, Gln – Glutamine, Arg – Arginine, Gly – Glycine, Glu – Glutamic acid, Asp – Aspartic acid, Cys – Cysteine, His – Histidine, Met – Methionine, Ser – Serine, Trp – Tryptophan, PCG – Protein-coding genes, RSCU – Relative synonymous codon usage, tRNAs – transfer RNAs, rRNAs – ribosomal RNA, BP – Bootstrap proportion, PP – Posterior probability.

RESULTS AND DISCUSSION

Genome organization and base composition

The present study is based on newly sequenced mitochondrial genomes of two species of moths of the family Erebidae, *H. bolinoides* and *L. dorsigera*. The complete mitogenome sequences of both species were deposited in GenBank with assigned accession numbers: *H. bolinoides* (GenBank Accession no: MW691121), *L. dorsigera* (GenBank Accession no: MW648384). The total length of the mitogenome of *H. bolinoides* is 15,640 bp and that of *L. dorsigera* 15,567 bp, which falls within the range of mitogenome lengths of previously sequenced noctuid moths (Fig. 1). Currently, the sequenced mitogenomes in the superfamily Noctuoidea range from 15,229 in *Helicoverpa gelotopoeon* to 16,346 bp in *Spodoptera frugipeda.* Both these species have metazoan mitogenomes composed of 13 protein-coding genes (PCGs); ATPase subunits 6 and 8 (*atp6 and atp8*), cytochrome c oxidase subunits 1–3 (*cox1, cox2 and cox3*), NADH dehydrogenase subunits 1–6 (*nad1, nad2, nad3, nad4, nad5* and *nad6*), subunit 4L nad (*nad4l*) and cytochrome B (*cob*), 22 transfer RNA (tRNA) genes; two ribosomal genes (*rrnL and rrnS*) and a control region (A+T-rich region). Four of the thirteen PCGs (*nad1, nad4, nad4l* and *nad5*) and eight tRNAs (*trnQ, trnC, trnY, trnF, trnH, trnP, trnL1* and *trnV*) and two rRNAs are encoded on the N-strand, whereas the other 23 genes (9 PCGs and 14 tRNAs) and the control-region (A+T-rich) are encoded on the J-strand (Table 1). All the genes are organized in a similar fashion without any rearrangement.

The nucleotide compositions of the two mitogenomes had a high A+T bias, of 81.77% in *H. bolinoides* and 80.17% in *L. dorsigera*. Among the noctuoid species for which mtDNA data is available, the lowest A+T content is 77.83% in *O. lunifer* and the highest 81.69% in *Gabala argentata* (Table S2). Both of the newly sequenced mitogenomes have a negative AT-skew on the majority strand and also a negative GC-skew (Table 2), as occurs in most other Noctuoid mitogenomes. The AT-skew values on the majority strand recorded for the two species of moths are, *H. bolinoides* (–0.017 and –0.073), *L. dorsigera* (–0.001 and –0.031) (Table 2), whereas the GC-skew ranges from (–0.125–0.330) in *H. bolinoides* and (–0.151–0.470) in *L. dorsigera* (Table 2). Similar patterns of nucleotide negative skew are also reported in the mitogenomes of other noctuoid taxa (Salvato et al., 2008; Sun et al., 2012; Wu et al., 2013, Zhu et al., 2018) (Table S2).

Table 1. Details of the genetic organization of the two newly determined *Noctuoidea mitogenomes*.

Name	Strand	Location	Length	Anticodon	Start	Stop	Intergenic nucleotides
Hypospila bolinoides							
nad4l	–	53–340	288		ATG	TAA	6
trnT	+	347–411	65	TGT			0
trnP	–	412–476	65	TGG			7
nad6	+	484–1014	531		ATT	TAA	97
cob	+	1112–2269	1158		ATG	TAA	45
trnS2	+	2315–2380	66	TGA			24
nad1	–	2405–3343	939		ATG	TAA	1
trnL1	–	3345–3412	68	TAG			–3
rrnL	–	3409–4811	1403				0
trnV	–	4812–4876	65	TAC			0
rrnS	–	4877–5612	736				88
trnM	+	6029–6096	68	CAT			0
trnI	+	6097–6162	66	GAT			–3
trnQ	–	6160–6228	69	TTG			58
nad2	+	6287–7300	1014		ATT	TAA	5
trnW	+	7306–7372	67	TCA			–8
trnC	–	7365–7427	63	GCA			0
trnY	–	7428–7492	65	GTA			47
cox1	+	7540–9075	1536		CGA	TAA	–5
trnL2	+	9071–9137	67	TAA			0
cox2	+	9138–9803	666		ATA	TAA	16
trnK	+	9820–9890	71	CTT			–1
trnD	+	9890–9956	67	GTC			0
atp8	+	9957–10121	165		ATC	TAA	–7
atp6	+	10115–10792	678		ATG	TAA	14
cox3	+	10807–11595	789		ATG	TAA	2
trnG	+	11598–11663	66	TCC			0
nad3	+	11664–12017	354		ATT	TAA	6
trnA	+	12024–12089	66	TGC			32
trnR	+	12122–12187	66	TCG			1
trnN	+	12189–12255	67	GTT			9
trnS1	+	12265–12330	66	GCT			24
trnE	+	12355–12421	67	TTC			6
trnF	–	12428–12494	67	GAA			3
nad5	–	12498–14235	1738		ATT	TAA	0
trnH	–	14236–14301	66	GTG			4
nad4	–	14305–15640	1336		ATG	T(AA)	
A+T-Rich Region		5701–5987					
Lygephila dorsigera							
nad4	–	1–1339	1338		ATG	T(AA)	9
nad4l	–	1349–1636	288		ATG	TAA	5
trnT	+	1642–1706	65	TGT			0
trnP	–	1707–1774	68	TGG			37
nad6	+	1812–2312	501		ATT	TAA	4
cob	+	2317–3471	1155		ATG	TAA	2
trnS2	+	3474–3540	67	TGA			21
nad1	–	3562–4500	939		ATG	TAA	0
trnL1	–	4501–4568	68	TAG			73
rrnL	–	4642–5893	1252				99
trnV	–	5993–6061	69	TAC			0
rrnS	–	6062–6856	795				0
trnM	+	7240–7307	68	CAT			0
trnI	+	7308–7371	64	GAT			–3
trnQ	–	7369–7437	69	TTG			54
nad2	+	7492–8505	1014		ATT	TAA	21
trnW	+	8527–8595	69	TCA			–8
trnC	–	8588–8655	68	GCA			14
trnY	–	8670–8734	65	GTA			3
cox1	+	8738–10273	1536		CGA	TAA	–5
trnL2	+	10269–10335	67	TAA			0
cox2	+	10336–11017	682		ATA	TAA	0
trnK	+	11018–11088	71	CTT			–1
trnD	+	11088–11159	72	GTC			0
atp8	+	11160–11324	165		ATA	TAA	–7
atp6	+	11318–11995	678		ATG	TAA	43
cox3	+	12039–12827	789		ATG	TAA	2
trnG	+	12830–12894	65	TCC			0
nad3	+	12895–13248	354		ATT	TAA	53
trnA	+	13302–13367	66	TGC			14
trnR	+	13382–13447	66	TCG			0
trnN	+	13448–13513	66	GTT			12
trnS1	+	13526–13591	66	GCT			11
trnE	+	13603–13670	68	TTC			3
trnF	–	13674–13743	70	GAA			9
nad5	–	13753–15471	1719		ATT	TAA	30
trnH	–	15502–15569	68	GTG			
A+T-Rich Region		6913–7177	265				

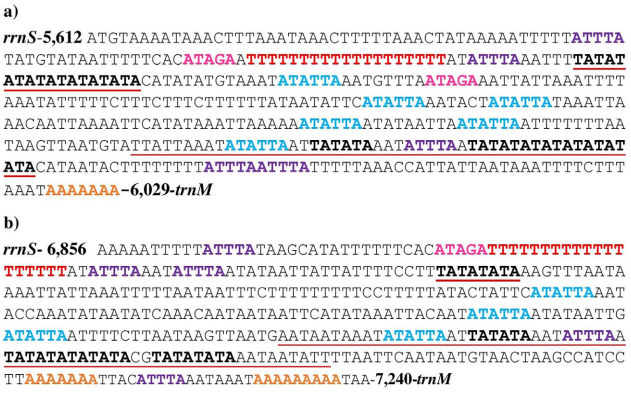

Fig. 2. Motifs and microsatellites in the A+T-rich region of the genomes of *H. bolinoides* and *L. dorsigera*. Motifs (ATAGA) are in bold pink, Poly-T stretch in bold red and Poly-A stretch in bold orange. Microsatellites (ATATTA) are in bold blue and microsatellites (ATTTA) in bold purple. All tandem repeats are underlined. Microsatellite (TA)3, (TA)4, (TA)6 and (TA)9 are shown in bold black underlined.

Protein-coding genes and codon usage

The mitogenomes of *H. bolinoides* and *L. dorsigera* consist of a complete set of PCGs as in other animal mitochondrial genomes. The total lengths of the 13 PCGs are 11,192 bp for *H. bolinoides* and 11,159 bp for *L. dorsigera,* accounting, for 71.56% and 71.22% of the entire mitogenomes, respectively (Table 2). Twelve of the thirteen PCGs have standard ATN start codons in *H. bolinoides* and *L. dorsigera*, except for *cox1,* which starts with the CGA codon in both of the species (Table 1). The non-canonical initiation codons for *cox1* are highly conserved in most insect groups (Cameron & Whiting, 2008; Liu et al., 2012). The stop codon TAA was recorded in almost all of the PCGs. Partial termination codons usually occur in insect mitogenomes, which may be related to post-transcriptional alteration during mRNA maturation (Ojala et al., 1981).

The relative synonymous codon usage (RSCU) values were calculated for five species belong to three families. Among them, the first two species are newly sequenced (Fig. S1). Based on the comparative analysis, the most frequently used codons are analogous in the two species. The most consistently recorded codons for *H. bolinoides* and *L. dorsigera* were UUU (Phe), UUA (Leu), AUU (Ile), AUA (Met), UAU (Tyr), AAU (Asn) and AAA (Lys); CGG (Arg) and GCC (Ala) were the least (Table S3).

Overlapping and intergenic spacer regions

In the case of overlapping and intergenic spacer regions, there were small intergenic spacers (IGS) ranging in size between 1 and 99 bp and totalling 495 bp in *H. bolinoides* and 519 bp in *L. dorsigera*. The longest intergenic spacer was located between *nad6* and *cob* with a length of 97 bp in *H. bolinoides* and between *rrnL* and *trnV* with a length of 99 bp in *R. privata.* In addition, six gene overlaps of 24 bp in *H. bolinoides* and five gene overlaps of 24 bp in *L. dorsigera* were recorded (Table 1).

Transfer RNA genes (tRNA)

The total lengths of the 22 tRNA genes was 1,463 bp in *H. bolinoides* and 1,484 bp in *L. dorsigera*, whereas individual tRNA genes typically range in size from 64 to 71 bp, among which eight tRNAs were encoded on the N-strand and the remaining 14 on the J-strand (Table 1). All the tRNA secondary structures of these two moths were inferred; among all the tRNA genes, 21 tRNAs could be folded into consistent clover-leaf secondary structures (Figs S2–S3). The *trnS1* (AGN) lacked a dihydrouridine (DHU) arm and formed a simple loop, which is reported in in several Lepidoptera (Liu et al., 2015, 2016; Sun et al., 2016; Huang et al., 2019). There were non-standard G-U, U-U mismatching pairs in the tRNA clover-leaf secondary structures of two Noctuoid mitogenomes. In total, 18 mismatches of 11 U-G and 7 G-U wobble pairs were recorded in the 14 tRNA genes of *H. bolinoides*, and 22 mismatches of 11 U-G, and 11 G-U wobble pairs in the 16 tRNA genes of *L. dorsigera*.

Ribosomal RNA genes

Two rRNA genes, *rrnL* and *rrnS*, were recorded in the newly sequenced mitogenomes. The *rrnL* and *rrnS* genes of *H. bolinoides* and *L. dorsigera* were situated between *trnL1* (CUN) and *trnV* and between *trnV* and the A+T-rich region, respectively (Table 1). In these two species, the length of the rrnL gene was 1,403 bp in *H. bolinoides* and 1,252 bp in *L. dorsigera*, while the rrnS gene was 736 bp in *H. bolinoides* and 795 bp in *L. dorsigera*. The A+T contents of the two rRNA genes were 85.13 in *H. bolinoides* and more or less equal in *L. dorsigera* (84.32%) (Table 2). The AT-skew and GC-skew values in both species were negative.

Table 2. Composition and skewness of the *H. bolinoides* and *L. dorsigera* mitogenomes.

	Size (bp)	A%	G%	T%	C%	AT%	AT skew	GC skew
H. bolinoides								
Whole genome	15640	39.28	7.33	42.49	10.89	81.77	–0.039	–0.176
PCGs	11192	38.76	8.2	41.35	11.71	80.08	0.031	–0.186
tRNA genes	1463	40.05	8.07	41.49	10.39	81.54	–0.017	–0.125
rRNA genes	2139	40.11	4.86	45.02	10.0	85.13	–0.057	–0.330
A+T-rich region	416	43.27	1.92	50.48	4.33	93.75	–0.073	–0.33
L. dorsigera								
Whole genome	15567	39.47	7.73	40.37	12.42	79.84	–0.011	–0.232
PCGs	11159	38.71	8.65	39.32	13.32	78.03	–0.007	–0.212
tRNA genes	1484	40.3	8.29	40.16	11.25	80.46	0.001	–0.151
rRNA genes	2047	41.62	4.98	42.7	10.7	84.32	–0.012	–0.364
A+T-rich region	383	44.13	2.35	47.0	6.53	91.13	–0.031	–0.470

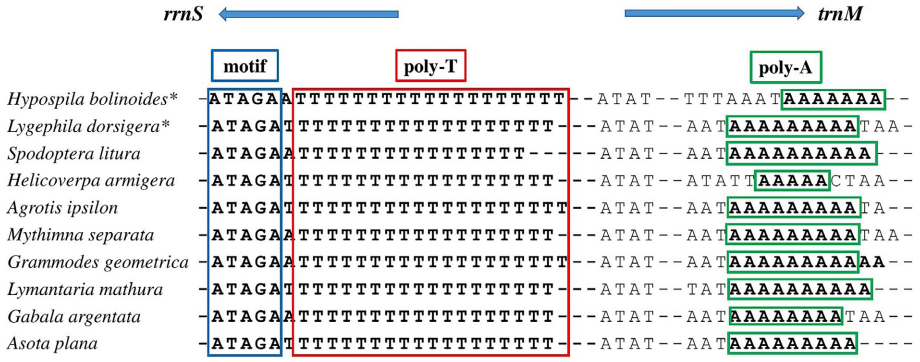

Fig. 3. Alignment of initiation site for A+T-rich region in the 10 species of Lepidoptera with completely sequenced mitogenomes. The blue colour boxed nucleotides indicate the conserved motif ATAGA. The red coloured boxed nucleotides indicate poly-T stretch and the green coloured boxed nucleotides indicate poly-A stretch. *Newly sequenced mitogenomes presented in this study.

The A+T-rich region

The A+T-rich regions in *H. bolinoides* and *L. dorsigera* were positioned amongst *rrnS* and *trnM* and were 287 bp and 383 bp in length, respectively (Table 1). The A+T-rich regions comprised 94.77% and 91.13% A+T content in *H. bolinoides* and *L. dorsigera*, respectively (Table 2). In the A+T-rich regions in the two Noctuoid species, an AT skew value of –0.073 (for *H. bolinoides*) and –0.031 (for *L. dorsigera*), and GC skew of –0.33 (*H. bolinoides*) and –0.470 (for *L. dorsigera*) were recorded for the two species. There are also tandem repeats in the A+T-rich region of the mitogenomes of *H. bolinoides* and *L. dorsigera,* with one tandem repeat of 50 bp in *H. bolinoides* and one of 64 bp in *L. dorsigera* (Fig. 5). In addition, there are three dinucleotides $(TA)_3$, 2 $(TA)_9$ and two motifs $(ATTTA)_5$, $(ATATTA)_6$, in *H. bolinoides* and four dinucleotides $(TA)_3$, $(TA)_4$, $(TA)_6$ and two motifs $(ATTTA)_5$ and $(ATATTA)_4$ in *L. dorsigera* (Fig. 2). The conserved "ATAGA+poly T" motif is situated downstream of *rrnS* in the A+T-rich regions of *H. bolinoides* and *L. dorsigera.* Moreover, poly-A stretches (7 bp in *H. bolinoides* and 9 bp in *L. dorsigera*) are situated upstream of *trnM* (Fig. 3).

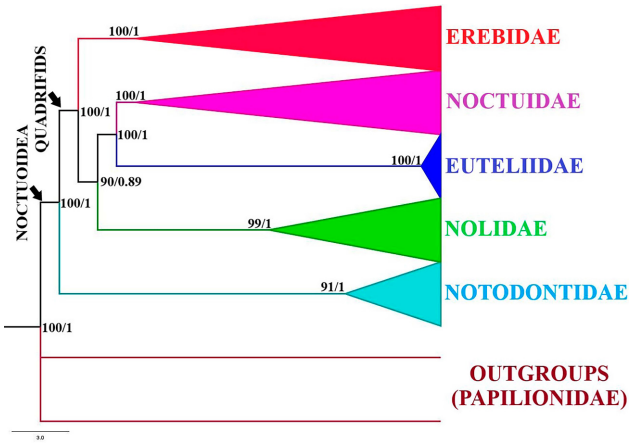

Fig. 4. Phylogenetic tree of the superfamily Noctuoidea based on maximum-likelihood analysis and Bayesian Inference. Clades representing families are coloured. Support values based on the two support measures (BP/PP) are shown next to the branches.

Phylogenetic relationships

The phylogenetic tree consists of five clades representing the five major Noctuoid families: Notodontidae, Nolidae, Euteliidae, Noctuidae and Erebidae (Fig. 4). This family topology, which is mostly based on mitogenomes, is congruent with previous morphological and molecular studies (Fibiger & Lafontaine, 2005; Lafontaine & Fibiger, 2006; Mitchell et al., 2006; van Nieukerken et al., 2011; Yang et al., 2015; Sun et al., 2020).

The present phylogenetic tree branches out from Erebidae, a major clade that includes two subclades with 86 species belonging to nine subfamilies: Arctiinae, Aganainae, Herminiinae, Erebinae, Toxocampinae, Calpinae, Scoliopteryginae, Lymantriinae and Hypeninae, with strong support (BP ≥ 100; PP = 1). The present analysis recovered Erebidae as a monophyletic group with maximum support (BP ≥ 100; PP = 1). Within the Erebidae clade, eight subfamilies were recovered nested in two major separate subclades with strong support (BP ≥ 100; PP = 1), and with moderate to high support among the subfamilies (Fig. 5a–b).

The first large subclade (56 species) includes the (Scoliopteryginae + (Calpinae + (Toxocampinae + Erebinae) + (Aganainae + Herminiinae) + Arctiinae)) with moderate support (BP ≥ 69; PP = 0.65). The tree topology indicates that Arctiinae have a sister relationship (BP ≥ 79; PP = 1), with a strongly supported pairing of Aganainae and Herminiinae (BP = 100; PP = 1). This triplet (Herminiinae + Aganainae) + Arctiinae) was recovered as a sister (BP ≥ 78; PP = 0.95) to a subclade (Calpinae + (Toxocampinae + Erebinae) (Fig. 5a–b).

In their respective groups, *Lygephila* (Billberg, 1820) and *Hypospila* (Guenée, 1852), the species, *H. bolinoides* and *L. dorsigera*, are the first to have complete mitogenome analyses. Currently there are no thorough phylogenetic analyses of each group. The present study therefore provides phylogenomic evidence for the newly sequenced species, which were previously classified based on morphological traits, are well placed in their respective subfamilies i.e., *H. bolinoides* (Noctuoidea: Erebidae: Erebinae) and *L. dorsigera* (Noctuoidea: Erebidae: Toxocampinae). Moreover, Toxocampinae is also established as a distinct subclade

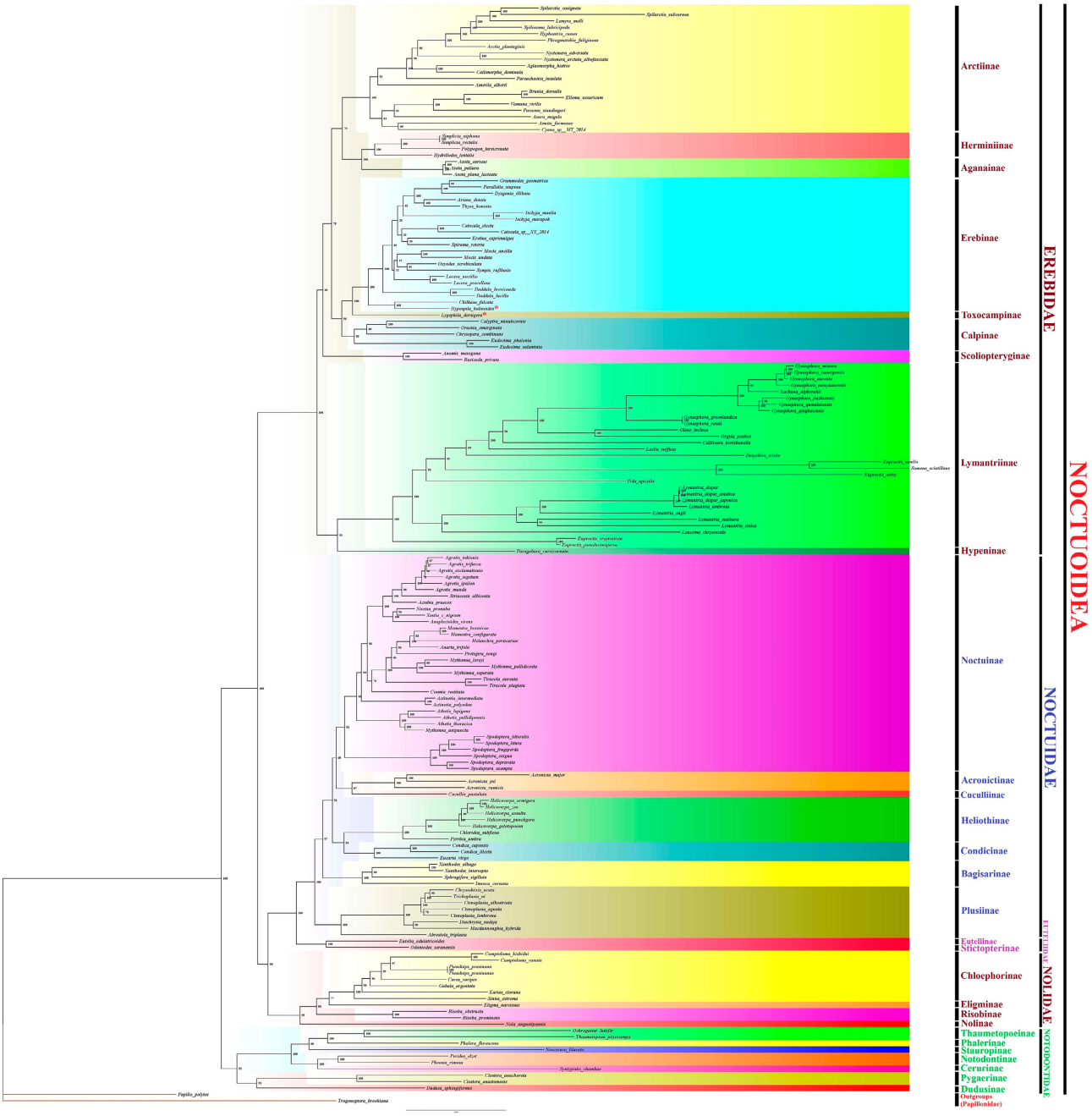

Fig. 5a. Phylogenetic tree of the superfamily Noctuoidea based on maximum-likelihood analysis using IQ-TREE.

and recovered as sister to subfamily Erebinae with strong support. (BP ≥ 100; PP = 1) (Fig. 5a–b).

The second subclade comprised of 30 species belonging to the subfamily Lymantriinae and Hypeninae, has high support values (BP ≥ 93; PP = 1). Moreover, Arctiinae is closely related to the subfamily Erebinae rather than Lymantriinae, since both subfamilies Arctiinae and Erebinae are clustered in the same clade. Erebidae clade as a whole showed the following relationship in both ML and BI analyses: (Hypeninae + Lymantriinae) + (Scoliopteryginae + (Calpinae + (Toxocampinae + Erebinae) + (Herminiinae + Aganainae) + Arctiinae)))) (Fig. 5a–b).

Another major clade with strong support (BP ≥ 99; PP = 1) and including 74 species represents three families; Noctuidae, Eutellidae and Nolidae. The independent Noctuidae clade of 60 species with strong support values (BP ≥ 100; PP = 1) has the relationship; (Plusiinae + (Bagisarinae) + (Condicinae + Heliothinae) + (Cucullinae + Acronictinae) + Noctuinae)). Some discrepancies and similarities were discovered when the results for the family Noctuidae were compared with those reported by Keegan et al. (2021), Regier et al. (2017), Zahiri et al. (2013) and Mitchell et al. (2006). According to the results presented here, the families Noctuinae, Heliothinae and Acronictinae, which make up the "higher noctuids", split from Plusiinae as an early diverging group. Eutellidae formed a separate clade and is placed sister to the family Noctuidae, with high support values (BP ≥ 98; PP = 1) and Nolidae is placed sister to the

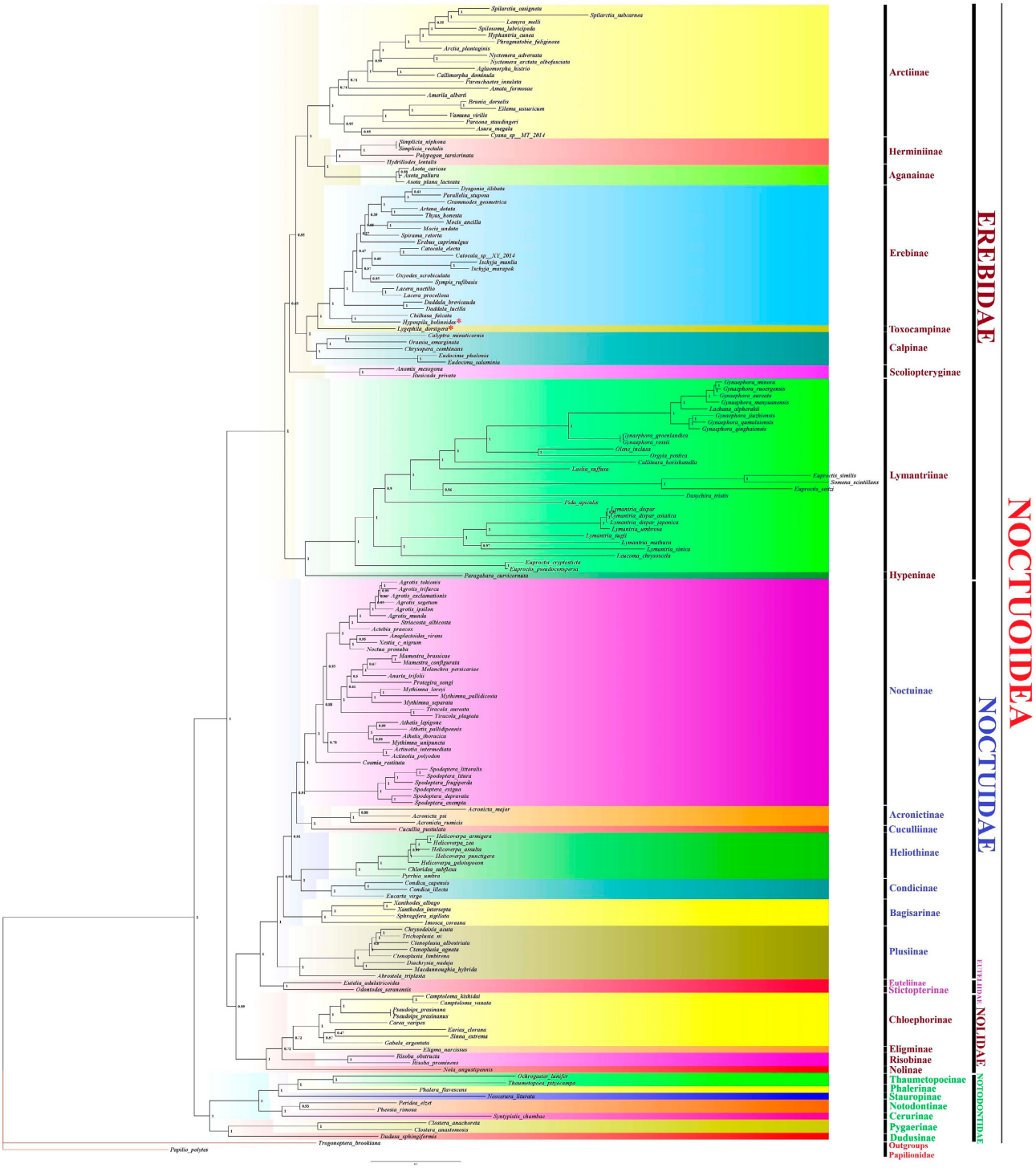

Fig. 5b. Phylogenetic tree of superfamily Noctuoidea based on Bayesian Inference using MrBayes.

pair of Euteliidae + Noctuidae, with high support values (BP ≥ 90; PP = 0.89). The relationship coming out of the Nolidae clade is: (Nolinae + (Westermaniinae + (Risobinae + (Eligminae + Chlophorinae)))) (Fig. 5a–b).

The family Notodontidae formed a separate clade (BP ≥ 100; PP = 1) that includes seven subfamilies; Thaumetopoeinae, Phlaerinae, Stauropinae, Notodontinae, Cerurinae, Pygaerinae, and Dudusinae, with strong support values (BP ≥ 100; PP = 1). The relationships were ((Dudusinae + Pygaerinae) + ((Cerurinae + Notodontinae + (Stauropinae + (Phlaerinae + Thaumetopoeinae))))) (Fig. 5a–b).

CONCLUSION

In this study, two complete Noctuoid mitogenomes were sequenced and 168 additional Noctuoidea mitogenomes and 2 butterfly mitogenomes were obtained from GenBank. These datasets were used to compare and contrast the mitogenomes, and analyse the evolutionary relationships within the superfamily Noctuoidea. These analyses

predicted the position of Notodontidae as sister to the other Noctuoid families (Erebidae, Nolidae, Euteliidae and Noctuidae). Moreover, the placement of Erebidae as sister to (Nolidae + (Euteliidae + Noctuidae)) agrees with the mitogenome analysis of owlet moths (Yang et al., 2015). As a sister group to Noctuidae, Euteliinae + Stictopterinae form a distinct and well-supported lineage, which is congruent with the results of Mutanen et al. (2010). These findings revealed the following relationships: (Notodontidae + (Erebidae + (Nolidae + (Euteliidae + Noctuidae)))). This result is congruent with the phylogenetic inferences of Yang et al. (2015). Huang et al. (2019), Sun et al. (2020, 2021) but not Zahiri et al. (2011).

Despite the fact that this evolutionary study offers a hypothesis for the Erebidae relationship, the results support the categorization of the Erebidae family. To fully comprehend and characterize the phylogeny of the superfamily Noctuoidea, additional research and samples are needed for a more thorough taxonomic and molecular categorization of the superfamily Noctuoidea up to tribal levels. As a result, concrete phylogeny requires more investigations, preferably based on a greater number taxa. Because of the enhanced systematic comparison of moths, this research provides a better understanding of the evolution and phylogeny of moths of the superfamily Noctuoidea.

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DECLARATION OF COMPETING INTERESTS. The authors declare no conflict of interest.

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