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MITOGENOME ANNOUNCEMENT

Complete mitochondrial genome of *Hypomecis punctinalis* Scopoli, 1763 and its phylogenetic position within family Geometridae

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ABSTRACT

Hypomecis punctinalis Scopoli, 1763 belongs to the Lepidopteran family Geometridae. We sequenced the complete mitochondrial genome (mitogenome) of *H. punctinalis*. The mitogenome is 15,648 bp long and contains a typical set of genes (13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes) and a 484 bp AT-rich region. All PCGs start with ATN codons and stop at TAA codon except for *cox1* using CGA as initiation codon and *nad4* and *nad5* using incomplete termination codon T. Within the mitogenome, 17 intergenic spacers and seven overlaps are founded. The intergenic nucleotides are 294 bp in total and two longest intervals locate between trn^{Gln} and *nad2* as well as trn^{Cys} and trn^{Tyr}. The overlap nucleotides are 47 bp in total and the maximum overlap lies between *cox2* and trn^{Lys}. The AT-rich region of the mitogenome contains an 'ATAGA + polyT' motif, three copies of 30-bp-repeat and a short polyA tail. The phylogenetic tree shows the relationships of four subfamilies of Geometridae are (((Ennominae + Geometrinae)+Larentiinae)+Sterrhinae)) and the relationships within subfamily Ennominae are ((((*Erannis*+*Biston*)+(*Jankowskia*+(*Hypomecis*+(*Apocheima* +*Milionia*))))+*Ectropis*)+*Abraxas*)+*Phthonandria*)+*Celenna*).

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Family Geometridae Leach, 1815 containing 2002 genera and 23,002 species is the second largest family in order Lepidoptera just after Erebidae (van Nieukerken et al. 2011). Geometrids are globally distributed and have been classified into six to nine subfamilies (Minet and Scoble 1998; Murillo-Ramos et al. 2019; Sihvonen et al. 2020). More interests have been focused on the molecular phylogeny of the family as well as the discovery of new species (Sihvonen et al. 2020; Wu et al. 2020; Yu and Wang 2020). Up to now, dozens of complete mitogenomes belonging to 17 genera and 20 species have been deposited in GenBank but not include genus *Hypomecis* Hübner, 1821. In this study, we report the complete mitogenome of *Hypomecis punctinalis* Scopoli, 1763 which is also called 'pale oak beauty' in some country and reconstruct its phylogeny within Geometridae.

The specimen of *H. punctinalis* was light-trapped from Xiangshan Mountain, Huaibei City, Anhui Province, China (116°48′34″ E, 33°59′1″ N). The total DNA was extracted from the muscle of the specimen legs according to the instruction manual of DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany) and the quantity and quality of the sampling DNA was evaluated by NanoDrop 2000c spectrophotometer (Thermo, Waltham, MA) and 1% agarose gel electrophoresis. The specimen (accession number 20180631027) and the DNA solution (accession number 20180631027DNA) deposited in the Specimens Room and the Human and Animal Genetics Laboratory (contact person Li Jun and email healthlicn@chnu.edu.cn), College of Life Science, Huaibei Normal University, China. Primers to amplify mitochondrial DNA were designed according to the conserve regions in mitogenomes of Lepidopteran insects and listed in supplemental table 1. The overlapping fragments were amplified using PrimeSTAR® GXL DNA Polymerase (Takara, Beijing, China). The complete mitochondrial DNA sequence was assembled using Lasergene DNASTAR kits and genes were annotated with MITOS and verified with NCBI BLAST (Bernt et al. 2013). Repeats discovered with Tandem Repeats Finder Program (Benson 1999).

The complete mitogenome of *H. punctinalis* (GenBank accession number MK903031) is 15,648 bp and the gene rearrangement is just like most of lepidopteran mitogenomes with trn^{Met}-trn^{Ile}-trn^{Gln} (Li et al. 2018). Twenty-three genes (nine protein-coding genes (PCGs) and 14 tRNAs) are located at the majority strand (J-strand) and 14 genes (four PCGs, eight tRNAs, and two rRNAs) at the minority strand (N-strand). In this mitogenome, 17 intergenic spacers (294 bp in total) and seven overlaps (47 bp in total) were founded. The intergenic nucleotides vary from 1 to 51 bp and two longest intervals locate between trn^{Gln} and *nad2* as well as trn^{Cys} and trn^{Tyr} while the overlap nucleotides vary from 1 to 20 bp and the maximum overlap lies between *cox2* and trn^{Lys}. All PCGs start with ATN codons except for *cox1* using CGA as initiation codon and all PCGs stop at TAA codon except for *nad4*

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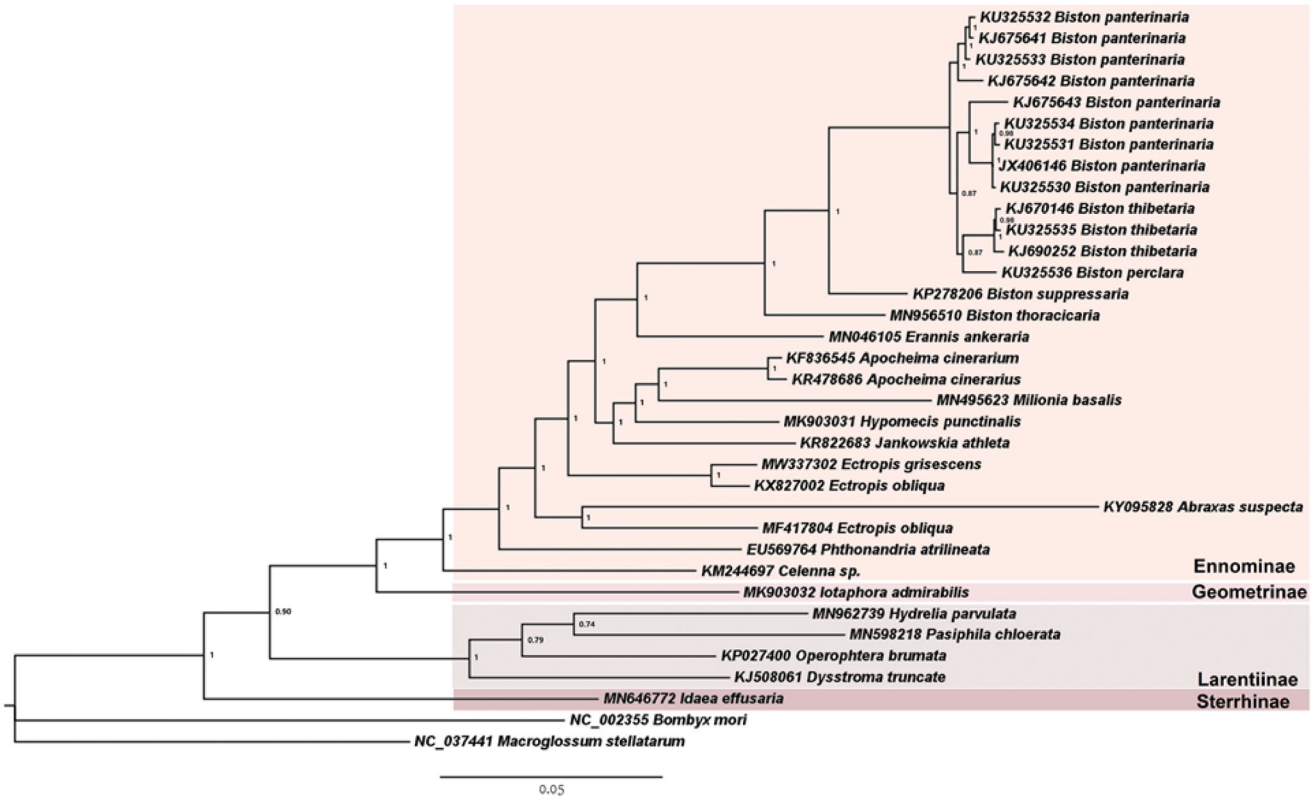

Figure 1. Phylogenetic tree for Geometridae based on all protein sequences with Bayesian inference (BI) method. The numbers at the nodes mean the Bayesian posterior probability. The scale bar indicates the number of nucleotide substitutions per site in the sequence. GenBank accession numbers of mitogenome sequences are listed before the scientific names of species.

and *nad5* using incomplete termination codon T. The mitogenome also has a 484 bp AT-rich region containing an 'ATAGA + polyT' motif, three copy repeats of 'ATTTATA TTAATTAATTTATTTAATGTAAT' and a polyA tail.

All protein sequences are incorporated to reconstruct phylogenetic tree using Bayesian inference (BI) method with the best-fit amino acid substitution model mtREV24 + G + I+F. The result shows the phylogenetic relationships of four subfamilies of Geometridae are (((Ennominae + Geometrinae)+Larentiinae)+Sterrhinae) and that within subfamily Ennominae are ((((*Erannis*+*Biston*) +(*Jankowskia*+(*Hypomecis*+(*Apocheima*+*Milionia*))))+*Ectropis*) +*Abraxas*)+*Phthonandria*)+*Celenna*) (Figure 1). Sterrhinae is sister to other subfamilies of Geometridae which is consistent with the result of the previous study based on one mitochondrial and 10 protein-coding nuclear gene regions (Murillo-Ramos et al. 2019). *Hypomecis punctinalis* is clustered into the clade of the subfamily Ennominae. The genus *Ectropis* is not a monophyletic group in our phylogenetic result. Since Geometridae has numerous members, more mitogenomes are needed to get more precise phylogenetic relationships.

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Depository: The specimen (accession number 20180631027) and the DNA solution (accession number 20180631027DNA) have been stored in the Specimens Room and the Human and Animal Genetics Laboratory college of Life Science, Huaibei Normal University, China (contact person Li Jun and email healthlicn@chnu.edu.cn).

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Data availability statement

The mitogenome sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov under the accession no. MK903031.

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