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

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Abstract

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

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

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

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

Gelechioidea, consisting of 1,425 genera and 16,250 described species worldwide, is one of the largest and the most important lepidopteran taxa (Hodges 1998). It includes some important insect pests and occupies a very important position in the evolutionary tree of Lepidoptera. The mitogenomes of Gelechioidea have rarely been studied, with only a few sequenced mitogenomes
available from five gelechioid species (09-2015), including four that are incomplete: Oegosoma novimundi (KJ508036), Perime dro sp. (KJ508041), Ethmia eupostica (KJ508047), and Endrosis sar citrella (KJ508037) (Timmermans et al. 2014), and a recently reported completely sequenced species, Promalactis suzukiella (Park et al. 2014). It seems that research on the phyoge ny of Gelechioidea had always had some divergence or some unresolved points (Passoa 1995; Kaila 2004, Kaila et al. 2011; Bucheli and Wenzel 2005, Regier et al. 2009, Mutanen et al. 2010).

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

**Materials and Methods**

**Sampling and DNA Extraction**

Larvae of B. macroscopa were collected from the field (an experimental field belonging to our laboratory and involving no endangered/protected species) around Hunan Agriculture University (Changsha, Hunan, China; 28°1’N, 113°4’E) and were bred in the laboratory with fresh water spinach (Ipomoea aquatica Forsk) leaves. Pupae of the second generation were collected and extracted to obtain total genomic DNA using the Wizard Genomic DNA Purification Kit (Promega, Beijing, China) according the manufacturer’s instructions.

**PCR Amplification and Sequencing**

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

PCR amplification was carried out in 25 μL reactions that contained 0.2 μL rTaq (TaKaRa Co., Dalian, China), 1 μL DNA, 2.5 μL 10× rTaq buffer (Mg2 + free), 2.5 μL 2.5 mM MgCl2, 2.0 μL dNTPs, and 0.5 μL of each primer. PCR conditions were 94°C for 5 min, 35 cycles at 94°C for 30 s, 50–59°C for 30 s, 72°C for 1–2.5 min, and a subsequent 10 min final extension at 72°C. The amplified fragments were sequenced directly by the appropriate primers using a commercial kit.

**Bioinformatic Analysis**

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

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

| Fragment | Region | Primer (J/N) | Primer sequence (J/F) 5′→3′ |
|----------|--------|--------------|---------------------------|
| F1       | rrnS-trnQ | SR-J-14610<sup>a</sup> | ATATAAGGTTACATTCTAGTAGT |
|          |        | Gln-Re<sup>b</sup> | GCACTATTCTTGATATATAGTTTA |
| F2       | nad2   | J-60<sup>d</sup> | GTATTTGGATGAGGATAGGAAA |
|          |        | N-735<sup>d</sup> | CAAATTAGGGGTTAATAGCCTTA |
| F3       | nad2-cox1 | J-489<sup>d</sup> | GGGGATATATAAACTCCCT |
|          |        | N-C1Re<sup>e</sup> | GCTGTGTAATAGTATTATAAATTTGATCATC |
| F4       | cox1-cox2 | C1-J-2167<sup>a</sup> | TTGGGATATATGGGGGTAATCCTC |
|          |        | C1-N-3649<sup>a</sup> | CCGCAAAATTTCGAAATTGACCA |
| F5       | cox2-nad3 | C2-J<sup>d</sup> | TTTGATATATGGGGGTAATCCTC |
|          |        | N-5731<sup>d</sup> | CCGCAAAATTCGAAATTGACCA |
| F6       | cox3-nad5 | C3-J-5470<sup>e</sup> | GCTGCAGCTGACATCCCTGAAAGT |
|          |        | N-5-7793<sup>d</sup> | AACCTTAAACATCATCACCT |
| F7       | nad5-nad4 | N-5-J-7572<sup>d</sup> | AAAAGGAAATTTTGAGCCTTTTGTAGT |
|          |        | N-4-N-9153<sup>d</sup> | TGAAGGTTATCACCAGACGG |
| F8       | nad4-cytb | N-4-J-8941<sup>e</sup> | GAAATGGCCGCCTCAATTGACG |
|          |        | N-11328<sup>d</sup> | GGCAAAATTGGAATATCATC |
| F9       | cytb-nad1 | CB-J<sup>d</sup> | CATATTGCAACCGAATTGAA |
|          |        | N-1-N-12588<sup>d</sup> | AATTGAACACCTTTTGGTATTTGT |
| F10      | nad1-rrnL | J-11876<sup>e</sup> | CGAGGTAAGTACACAAGACTCA |
|          |        | 16S-N<sup>d</sup> | ATATGTCATATTGGCGCCGTC |
| F11      | rrnL-rrnS | J-13900<sup>d</sup> | CTTGGTGTACAGAATTGATTA |
|          |        | N-12SR<sup>d</sup> | GTAAAAGTTCAAATAGCAAG |

<sup>a</sup>Primers modified from Simon et al. (1994) prior to this mitogenome.
<sup>b</sup>Primers modified from Lee et al. (2006) prior to this mitogenome.
<sup>c</sup>Primers from Simon et al. (2006) prior to this mitogenome.
<sup>d</sup>Primers newly designed for this genome.
The comparative analysis and spread correction were performed by the software Mega 6.0 (Tamura et al. 2013) to obtain the complete mitogenome of *B. macroscopa* (Tamura et al. 2013). The related lepidopteran sibling species were analyzed by blast searches on the NCBI database. The skew of the analyzed species was made using the formulas: AT skew = -(A - T)/(A + T); GC skew = (G - C)/(G + C) (Junqueira et al. 2004).

Phylogenetic Analysis
Along with the *B. macroscopa* mitochondrial genome, 29 available lepidopteran species (Son and Kim 2011, Gong et al. 2012) and four hepatopanermitogones (*Napalus hunanensis*, *Thitarodes pui*, *Abamas yunnanensis*, and *Thitarodes renzhiensis*) (Cao et al. 2012, Yi et al. 2016a, 2016b) were used in the phylogenetic analysis (Table 2). The nucleic acid regions and amino acid sequences from all 13 PCGs were aligned using Mega 6.0 (Tamura et al. 2013). Gblock 0.91b with default settings was used with conserved regions of the likelihood (ML) method was conducted with RAxML v 8.0.2 generations and a burn-in series of 1,000. In addition, the maximum (Darriba et al. 2012), with the MCMC analysis run for 1,000,000 Bayesian inference (BI) analysis was performed via MrBayes v3.2.3.

The 13 individual best fitting models for the nucleic acid dataset were defined with the Akaike Information Criterion (AIC) and the putative amino acids (Castresana, 2000).

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

The intergenic spacer sequence was 184 bp long in total and was made up of 16 regions, which varied from 1 to 83 bp in length, with two major intergenic spacer sequences with 65.8% of base pairs of the entire spacer regions located between trnQ and nad2 (83 bp), and ccox3 and tRNA (38 bp). In addition, 10 overlaps of 48 bp in length were present in the *B. macroscopa* mitogenome. The smallest was only 1 bp, and the largest was just 10 bp, located between nad1 and trnH.

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

### Results

Genome Organization and Base Composition

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### Table 2. Source and information for the phylogenetic analysis

| Superfamily | Family | Species | Accession number | Length (bp) | References |
|-------------|--------|---------|-----------------|-------------|------------|
| Urodoidea   | Urodidae | Urodus decens | KJ508062 | 15,279 | Timmermans et al. (2014) |
| Yponomeutoidea | Plutellidae | Plutella xylostella | NC_025322 | 16,014 | Dai et al. (2016) |
| Yponomeutoidea | Yponomeutidae | Prays oleae | NC_025948 | 16,499 | van Asch et al. (2016) |
| Tineoidea   | Tineidae | Tineola iniellia | KJ508045 | 15,661 | Timmermans et al. (2014) |
| Zygaoidea   | Zygaoidea | Rhodopsona rubiginosa | KM244668 | 15,248 | Tang et al. (2014) |
| Tortricoidea | Tortricidae | Adoxophyes bommae | NC_008141 | 15,680 | Lee et al. (2006) |
| Genchoidea  | Genchoidea | Grapholita dimorpha | NC_014299 | 15,368 | Zhao et al. (2011) |
| Acrididae   | Acrididae | Choristoneura longicellana | NC_019996 | 15,759 | Unpublished |
| Elachistidae | Elachistidae | Acleris fimbriata | NC_018754 | 15,933 | Zhao et al. (2014) |
| Cosmopterigidae | Epiphasy postitattana | Grapholita molesta | NC_014806 | 15,717 | Son and Kim (2011) |
| Gelechiidae | Gelechiidae | Spilonota lechriaspis | NC_012499 | 15,368 | Zhao et al. (2011) |
| Gelechiidea  | Oecophoridae | Choristoneura longicellana | NC_019996 | 15,759 | Unpublished |
| Elachistidae | Epiphasy postitattana | Grapholita molesta | NC_014806 | 15,717 | Son and Kim (2011) |
| Cossoidea   | Cossidae | Oegysita hippophaeacolus | NC_023936 | 15,431 | Gong et al. (2013) |
| Hepialoidea | Hepialidae | N. hunanensis | NC_024424 | 15,301 | Yi et al. (2016a) |
| Gracillarioidea | Gracillariidae | Phyllomycter platani | NC_023530 | 15,064 | Yi et al. (2016b) |
| Copromorphoidea | Carposinidae | Carposina sasaku | NC_023212 | 15,611 | Wu et al. (2016) |
were obviously distinct with an AT skew value of 0.014 and GC skew value of −0.188.

**Codon Usage in PCGs**

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

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

**The Secondary Structure for RNA Genes**

The two rRNA genes, 16s (*rrnL*) and 12s (*rrnS*), were 1,457 and 778 bp in length, respectively. The *rrnL* gene was situated in *tRNA(UUC)* and *trnV*, and the *rrnS* gene was located between *trnV* and the A+T-rich region. Both of these genes were located on the
The complete secondary structures of the *rrnL* and *rrnS* molecules were determined and are shown in Figs. 3 and 4, respectively. The *rrnL* secondary structure contained six domains, made up of two conserved and four variable regions, and three other domains appeared in the *rrnS* secondary structure. Twenty-two tRNA genes, ranging from 63 to 71 bp in length, were contained in the *B. macroscopa* mitogenome. Fourteen of these genes were mapped on the J-strand and eight were mapped on the N-strand. Typical cloverleaf secondary structures were also found in this species, except for trnS(UCN) which lacked a dihydrouridine arm (Fig. 5).

### Features in the A + T-Rich Region

The A + T-rich region, located between the trnM and *rrnS* genes, was 325 bp long with a higher content (96.7%) and the absence of a large repeating fragment. The conserved structure consisted of an ‘ATAGT’-like motif, and included a poly-T. The poly-T was considered to be the origin of the minor strand replication (Fig. 6A). Three microsatellites, ‘(TA)$_6$’, ‘(TA)$_9$’, and ‘(TA)$_7$’, were observed in this region, and they were located 205, 241, and 261 bp upstream of *rrnS*, respectively. The conserved ‘ATTTA’ sequence was located between the microsatellite (TA)$_6$ and (TA)$_9$. A potential stem-loop structure existed in the A + T-rich region without the ‘TATA’ sequence at the 5' end and without the ‘G(A)nT’ at the 3' end (Fig. 6B).

### Phylogenetic Relationships

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

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

| Gene   | Strand | Nucleotide no. | Size(bp) | IN | Anticodon | Start codon | Stop codon |
|--------|--------|----------------|----------|----|-----------|-------------|------------|
| trnM   | J      | 1–67           | 67       | 8  | CAT       |             |            |
| trnL   | J      | 76–142         | 67       | 4  | GAT       |             |            |
| trnQ   | N      | 147–214        | 70       | 83 | TTA       |             |            |
| nad2   | J      | 298–1,284      | 987      | –2 | ATA       | TAA         |            |
| trnW   | J      | 1,283–1,350    | 68       | –8 | TCA       |             |            |
| trnC   | N      | 1,343–1,409    | 67       | 11 | GCA       |             |            |
| trnY   | N      | 1,421–1,489    | 69       | 3  | GTA       |             |            |
| cox1   | J      | 1,493–3,028    | 1536     | –5 | CGA       | TAA         |            |
| trnL(UCR) | J   | 3,024–3,091    | 68       | 0  | TAA       |             |            |
| cox2   | J      | 3,092–3,778    | 687      | –5 | ATG       | TA-         |            |
| trnK   | J      | 3,774–3,844    | 71       | 0  | CTT       |             |            |
| trnD   | J      | 3,845–3,913    | 69       | 9  | GTC       |             |            |
| atp8   | J      | 3,923–4,078    | 156      | –7 | ATA       | TAA         |            |
| atp6   | J      | 4,072–4,749    | 678      | –1 | ATG       | TAA         |            |
| cox3   | J      | 4,749–5,337    | 789      | 38 | ATG       | TAA         |            |
| trnG   | J      | 5,576–5,643    | 68       | –3 | TCC       |             |            |
| nad3   | J      | 5,641–5,997    | 357      | 2  | ATA       | TAA         |            |
| trnA   | J      | 6,000–6,067    | 67       | –1 | TGC       |             |            |
| trnR   | J      | 6,067–6,130    | 64       | 0  | TCG       |             |            |
| trnN   | J      | 6,131–6,196    | 66       | 3  | GTT       |             |            |
| trnS(AGN) | J   | 6,200–6,265    | 66       | 7  | GCT       |             |            |
| trnE   | J      | 6,273–6,335    | 63       | 1  | TTC       |             |            |
| trnF   | N      | 6,337–6,402    | 66       | 1  | GAA       |             |            |
| nad5   | N      | 6,404–8,146    | 1743     | –10| ATT       | TAA         |            |
| trnH   | N      | 8,137–8,201    | 65       | 0  | GTG       |             |            |
| nad4   | N      | 8,202–9,540    | 1339     | 0  | ATG       | T-          |            |
| nad4L  | N      | 9,541–9,834    | 294      | 2  | ATG       | TAA         |            |
| trnT   | J      | 9,837–9,902    | 66       | 0  | TGG       |             |            |
| trnP   | N      | 9,903–9,972    | 70       | 2  | TGG       |             |            |
| nad6   | J      | 9,975–10,502   | 528      | 14 | ATA       | TAA         |            |
| cytB   | J      | 10,517–11,671  | 1155     | 2  | ATG       | TAA         |            |
| trnS(UCN) | J  | 11,674–11,743  | 70       | 3  | TGA       |             |            |
| nad1   | N      | 11,747–12,703  | 957      | –6 | ATA       | TAG         |            |
| trnL(CLIN) | N | 12,698–12,768  | 71       | 16 | TAG       |             |            |
| trnL   | N      | 12,769–14,225  | 1457     | 0  |           |             |            |
| trnV   | N      | 14,226–14,291  | 66       | 0  | TAC       |             |            |
| rrnS   | N      | 14,292–15,069  | 778      | 0  |           |             |            |
| A + T-rich | – | 15,070–15,394  | 325      | 0  |           |             |            |
polyphyletic group. Tortricoidea were recovered as a sister to the rest of Apoditrysia in the analyses, and this was supported by a bootstrap value of 100% and a posterior probability of 1.00. The Gelechioidea was assumed to be a sister group to the Apoditrysia (Cho et al. 2011), but it was not supported in our results because the Gelechioidea was nested within the clade Apoditrysia in the two trees. The Apoditrysia group (Gelechioidea, Zygaenoidea, Cossoidea, Copromorphoidea, Urodoidea, and Tortricoidea) was a monophyletic clade with a bootstrap value of 100% and a posterior probability of 1.00. A sister relationship between Yponomeutoidea + Gracillarioidea and the Apoditrysia was supported by a bootstrap value of 88% and a posterior probability of 1.00. The superfamily Tineoidea was a monophyletic clade with a bootstrap value of 100% and a posterior probability of 1.00. This is the earliest clade derived from Ditrysia, supporting the general agreement that tineoids are the oldest ditrysian superfamily (Minet 1991, Kristensen and Skalski 1998). The superfamily Gelechioidea, which includes O. novimundi, E. sarcitrrella, E. eupostica, B. macroscopa, and Perimede sp. in this study, formed a monophyletic group supported by a posterior probability of 1.00 (Fig. 8).

Discussion

Genome Organization and Base Characteristics

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

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

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

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

Protein-Coding Genes

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

Eleven of the 13 PCGs ended with ‘TAA’ codons, while the remaining two stopped with ‘TTA’ or a single ‘T’. The incomplete
Fig. 4. Predicted secondary structure of the \textit{rrnS} gene in the \textit{B. macroscopa} mitogenome. Red-colored letters stand for variable positions and blue for conserved positions. Tertiary structures are noted by continuous lines. Base-pairing is delineated as follows: Watson-Crick pairs are joined by lines, GU pairs by dots, and other non-canonical base pairs by asterisks. Each domain is indicated with Roman numerals.
Fig. 5. Predicted secondary structure of tRNAs gene in the *B. macroscopa* mitogenome. Dashes (–) indicate Watson-Crick base-pairing, centered solid pentagram (pentagram) indicate G-U base-pairing, and solid triangles (~) indicate mismatches.
termination codons could be completed by the mRNA process of polyadenylation (Anderson et al. 1981). It is important that the incomplete stop codons could make up the complete TAA in the assembly process of mRNA (Boore 1999, Gong et al. 2012). It was concluded that A and U were more frequently used in PCGs because the value for the RSCU of NNU and NNA codons was always >1.

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

Phylogenetic Relationships
Knowledge of insects involves insect ecology, behavior, systematics, host plant choices, and so on, but information about the worldwide superfamly Gelechioidea has been very limited (Hodges 1998). The sister group of Gelechioidea is still unknown (Mutanen et al. 2010). Mitogenomic phylogeny research was carried out by Timmermans et al. (2014), and it demonstrated that the Gelechioidea was not grouped with other lower Apoditrysia. The phylogenetic systematics of 10 superfamilies in Lepidoptera were constructed based on mitochondrial data that showed a sister relationship between Gelechioidea and Zygaenidae + Cossoidea, because both of them cluster in a same node and have a positive Bayesian posterior probability of 1.00. Significantly, our study may support the Gelechioidea belonging to the Apoditrysia, and it differed from the findings of Timmermans et al. (2014). The two analyses were both conducted based on mitogenome data, and whether these differences were due to the introduction of the
complete mitogenome in this study remains to be investigated. The phylogenetic relationship among the Yponomeutoide, Gracillarioidea, Gelechioidea, and Apoditrysia were not well resolved by Kristensen et al. (2007). In our results, the Yponomeutoide + Gracillarioidea and Apoditrysia were sister groups and clustered on the same branch, with a bootstrap value of 88% and a high posterior probability of 1.00. Although the value of ML was lower, it can still provide some information for the uniform relationships. The structure was supported by Regier et al. (2013), and it had many differences from the nested Yponomeutoide + Gracillarioidea and Gelechioidea (Kristensen and Skalski 1998).

The ML and BI analyses have different advantages in reconstructing phylogenetic trees, and in some instances the results may be different according to the different methods. The results of this study, which revealed the monophyly of Gelechioidea, was mostly supported by previous analyses that were based on morphology, ecology, and mitogenomes (Passoa 1995, Kaila 2004, Bucheli and Wenzel 2005, Regier et al. 2009, Mutanen et al. 2010, Kaila et al. 2011). Although the ML value was lower, the two separate analyses correctly interpreted the monophyly of Gelechioidea. Six species were chosen, representing five subfamilies (Symmocinae, Oecophoninae, Ethmiinae, Dichomeridinae, and Chrysopeleiinae). According to the analysis of Mutanen et al. (2010), Autostichidae was not monophyletic unless Glyphydoceridae and Deoclonidae were included. The tree, in Figs. 7 and 8, showed that Autostichidae and Oecophoridae clustered in the same node, and therefore the monophyly of Autostichidae was not confirmed. With further observations, the Ethmiinae and the other two subfamilies had higher bootstrap values and strongly resembled the earlier results of phylogenetic relationships indicated by Passoa (1995) and Bucheli and Wenzel (2005). The consensus on familial relationships ((Autostichidae + Oecophoridae) + (Gelechiidae + Cosmopterigidae)) by ML and ((Elachistidae + Gelechiidae) + Oecophoridae) by BI obviously conflicted with the latest molecular analysis that revealed different affinities (Elachistidae + (Cosmopterigidae + Gelechiidae) + (Autostichidae + Oecophoridae)) (Hentikka et al. 2014). The challenge of getting better mitogenome-based phylogenies probably requires that more Gelechioidea mitogenomes are sequenced.
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Fig. 8. BI phylogram using the concatenated 13 PCGs of mitogenomes obtained in these species, which used partitioned models for analysis. The scale bar indicates the number of substitutions per spot, and values of each node specify bootstrap percentages of 1,000 replicates.
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