The Complete Mitochondrial Genome of the Plant Bug *Lygus pratensis* Linnaeus (Hemiptera: Miridae)

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Abstract

*Lygus pratensis* is a phytophagous pest responsible for yield losses in Bt alfalfa and other economic crops in Northwestern China. To better characterize Miridae at the genomic level, the complete mitochondrial (mt) genome of *L. pratensis* was sequenced and analyzed in this study. The mt genome was amplified via the polymerase chain reaction to generate overlapping fragments. These fragments were then sequenced, spliced, and analyzed to include the examination of nucleotide composition, codon usage, compositional biases, protein-coding genes (PCGs), and RNA secondary structures. Phylogenetic relationships between *L. pratensis* and other species in different Heteroptera families were also examined. The mt genome was found to be a typical circular genome with a length of 16,591 bp and a total AT content of 75.1%, encoded for 13 PCGs, 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (lrRNA and srRNA), and a noncoding control region. The nucleotide composition of the entire mt genome was heavily biased toward A and T. All of the tRNAs were predicted to have classic clover leaf structures, but three of the tRNAs (lrRNA and srRNA), and a noncoding control region. The control region (2,017 bp), which was found to be located between 12S and lrRNA, contained three tandem repeat elements. Phylogenetic analyses showed that *L. pratensis* is closely related to the other three examined *Lygus* bugs, and that it is a sister group to *Apolygus* and *Adelphocoris*. This study confirms the usability of the mt genome in phylogenesis studies pertaining to the *Lygus* genus, within Miridae.

Key words: *Lygus pratensis*, mitogenome, phylogenetic relationship, Miridae

Miridae, which belongs to the order Hemiptera, is one of the most diverse families of insects and covers over 1,200 genera and 11,000 species (Cassis and Schuh 2012). Some of species have been associated with serious economic losses of Bt crops in developed and developing countries, alike, including the United States (Bauer et al. 2006), Australia (Fitt et al. 1994), Canada (May et al. 2003), and China (Guo et al. 2005, Wu and Guo 2005, Lu et al. 2008). *Lygus pratensis* Linnaeus, which is the most common species found in Northwestern China, is one of five species affecting Bt crops in China (Lu et al. 2010). In recent years, *L. pratensis* has adversely affected alfalfa and other economic crops, and hindered the expansion of the dairy industry in Northwestern China. At present, very few studies have reported on the ecology, phenology, or management of this pest (Li et al. 2007b, Lu et al. 2010). Furthermore, no molecular markers have been used to investigate *L. pratensis* population genetics structure or evolutionary patterns, with this insight potentially facilitating forecasting and management protocols.

In insects, mitochondrial (mt) genomes have been extensively studied for marker discovery (Cameron 2014). The insect mt genome consists of a circular, double-stranded molecule that is approximately 14–20 kb and contains a highly conserved exon arrangement comprising 37 genes. The insect mitogenome commonly includes 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and 2 ribosomal RNA genes rRNAs, srRNA (12S), and lrRNA (16S) (Boore 1999, Taanman 1999, Cameron 2014). Additionally, the genome contains a major noncoding region known as the A+T-rich region that plays a role in the initiation of DNA replication and transcription (Clayton 1992, Wolstenholme 1992). In recent years, the numbers of complete insect mt genomes have increased. mt genomes are of interest due to their small size, rapid evolution, maternal inheritance, low rate or lack of recombination, and relatively conserved gene content and organization (Gissi et al. 2008, Timmermans et al. 2010). These genomes are widely used as a maternal molecular marker for taxonomy, molecular phylogenetics, population genetics, and phylogeography (Hwang et al. 2001, Ma et al. 2009, Chen et al. 2012). However, phylogenetic analysis based on the current taxon has been limited, thus potentially restricting the usefulness of an mtDNA genome based phylogenetic study (Zhao et al. 2015). Although researchers...
have conducted some phylogenetic research on Miridae, studies have mainly focused on morphological analyses, with little molecular evidence presented (Schuh et al. 2009).

At present, the *L. pratensis* mt genome has not been previously reported. Herein, the complete *L. pratensis* mitogenome was cloned, sequenced, identified, and analyzed. Moreover, gene annotations, mitogenomic structural analysis, nucleotide composition, codon usage, tRNA secondary structure, noncoding and control region. Analyses were also performed. The research presented herein will offer a foundation for examining *L. pratensis* molecular evolution and provide further insight into the phylogenetic relationships among Hemiptera species.

**Materials and Methods**

**Specimen Collection**

Adult *L. pratensis* species were collected from alfalfa fields in the Helin County Experimental Station at the Institute of Grassland Research (40°60′N, 111°80′E), Chinese Academy of Agricultural Science (Hohhut, Inner Mogolian Province, China) in May 2016. All of the samples were quickly preserved in 100% ethanol at −20°C until arrival at the Inner Mongolia Agricultural University (40°48′N, 111°42′E).

**DNA Extraction, PCR Amplification, and Sequencing**

Genomic DNA was extracted from eight adults using a DNeasy DNA Extraction kit (AiDeLai Biotech. Co. Ltd, Beijing, China) and mtDNA amplified via the polymerase chain reaction (PCR). Primers were designed to target generally conserved regions and amplified short fragments from 16s, 12s, *cox1*, *atp6*, *cox3*, *nad4*, *cytB*, and *nad* (Table 1). The PCR reaction was carried out with LA Taq polymerase (Takara, Japan) for 35 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min/1 kb for the extension. The final MgCl₂ concentration in the PCR reaction was 2.0 mmol/liter. The obtained amplicons were then purified, cloned into pMD18-T vectors (Takara Dalian Co., Ltd., Dalian, China), and sequenced.

**Sequence Analysis, Annotation, and Secondary Structure Predictions**

The sequencing data were assembled using BioEdit software as previously described (Hall 1999). Protein-coding regions were identified using ORF Finder on the NCBI website and PCG nucleotide sequences were translated based on invertebrate mtDNA sequences. Sequence alignment was carried out using ClustalX (Thompson et al. 1997). Composition skew analysis was performed to examine base composition, with asymmetry measured according to the formulas (AT skew = [A−T]/[A+T] and GC skew = [G−C]/[G+C]) (Perna and Kocher 1995). MEGA 6.0 was used to calculate base composition and codon usage.

Transfer RNA genes and their predicted secondary structures were identified using tRNAscan-SE (http://lowelab.ucsc.edu/tRNAscan-SE/), with a few of the tRNA genes identified by comparing sequence homologies with tRNAs from other published plant bugs. Two rRNA genes were identified by Blastn on the NCBI website and

| Table 1. Primers used for amplifying the complete mt genome |
|-------------------------------------------------------------|
| **Fragment** | **Primer name** | **Nucleotide sequences (5′→3′)** | **Sequence size (bp)** | **Starting site–stop site** |
| 1 | LLF1 | ACTAAATCCGTAGAGATC | 973 | 82–1055 |
| 2 | LLR1 | CGAAAGTGTCACTATTGATG | 1,069 | 891–1960 |
| 3 | LLF2 | GAATAACAGAAAAGTTATC | 1,088 | 1,796–2,904 |
| 4 | LLR2 | CAGTATATGAATGTTCAGC | 1,300 | 2,708–4,008 |
| 5 | LLF3 | GATTACCCTGATAGATTC | 970 | 6,746–7,716 |
| 6 | LLR3 | TCCCTTTAGGTGGTCATAG | 1,171 | 3823–4,994 |
| 7 | LLF4 | GTATACTCTTCGATAGAC | 1,242 | 5670–6,911 |
| 8 | LLR4 | ATCGGTTGTCGTCGCCGTC | 1,051 | 4,803–5,854 |
| 9 | LLF5 | CCCAATTTGTAGAAAAC | 970 | 6,746–7716 |
| 10 | LLR5 | TACCCCTATACATCTATC | 1,261 | 8,754–10,015 |
| 11 | LLF6 | GTGACGAGAATATCGAG | 1,051 | 4,803–5,854 |
| 12 | LLR6 | GTATGTTTAAATACATCC | 1,171 | 3823–4,994 |
| 13 | LLF7 | TTCTATCTTCGATAGAC | 1,242 | 5670–6,911 |
| 14 | LLR7 | TCCCCCTACTCATCATC | 1,261 | 8,754–10,015 |
| 15 | LLF8 | TTCTATCTTCGATAGAC | 1,242 | 5670–6,911 |
| 16 | LLR8 | TCTGTATATGAATGTTCAGC | 1,051 | 4,803–5,854 |
| 17 | LLF9 | TTCTATCTTCGATAGAC | 1,242 | 5670–6,911 |
| 18 | LLR9 | TTCTATCTTCGATAGAC | 1,242 | 5670–6,911 |
| 19 | LLF10 | TTCTATCTTCGATAGAC | 1,242 | 5670–6,911 |
| 20 | LLR10 | TTCTATCTTCGATAGAC | 1,242 | 5670–6,911 |
then validated by performing a homologous gene alignment against other plant bugs. Furthermore, the entire control region was examined for tandem repeats using the Tandem Repeats Finder program (Benson 1999).

**Phylogenetic Analyses**

Phylogenetic analyses were performed based on 14 complete representative bug mt genomes from GenBank, and 11 Miridae species as follows: *Lygus hesperus* (NC_024641), *Lygus lineolaris* (NC_021975), *Lygus rugulipennis* (KJ170898), *L. pratensis* (MF188235), *Lygus lucorum* (NC_023083), *Adelphocoris fasciaticollis* (KJ001714), *Adelphocoris lineolaris* (KJ020286), *Adelphocoris nigritulus* (KJ020287), *Adelphocoris suturalis* (KJ020288), *Trigonotylus caelestialium* (KJ170899), and *Nesidiocoris tenuis* (NC_022677). The remaining species belonged to the Anthocoridae, Reduviidae, and Nabidae families in Hemiptera and included *Orius niger* (NC_012429), *Agriosphodrus dohrni* (NC_015842), and *Gorpis annulatus* (NC_019595). DNA alignments were inferred based on sequences from all 14 of the PCGs and concatenated using ClustalX. Phylogenetic relationships were examined using the neighbor joining (NJ), maximum likelihood (ML), and Bayesian inference (BI) methods. An NJ tree was constructed using MEGA 5.0 with 1,000 bootstrap replicates. ML analysis was conducted using RAxML 7.0 (Stamatakis 2006), with an MrREV model for amino acid sequences used to optimize the topology. Node support was calculated by acquiring bootstrap values from heuristic searches of 1,000 resampled data sets, using the rapid bootstrap feature in RAxML (Stamatakis et al. 2008). BI analysis was carried out using MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003), with two simultaneous runs of 1,500,000 generations conducted for the majority of the PCGs, 77.5% in tRNA genes, 79.1% in rRNA genes, and 72.2% in the control region (Table 3). When performing skew statistics on the PCGs, it was clearly shown that PCGs on the J strand were TA-skewed, while the N strand was AT-skewed.

The nucleotide AT bias was also reflected in the codon usage. When analyzing the base composition of 13 PCGs, the relative synonymous codon usage (RSCU) values showed that at the third codon position, A or T appeared substantially more often than did G or C (Fig. 2). While the four most used codons included TTT (F), TTA (L), ATT (I), and ATA (I).

**Base Composition and Codon Usage**

The nucleotide composition of the *L. pratensis* mt genome was heavily biased toward A (42.7%) and T (32.4%), while much lower levels were seen for G (10.3%) and C (14.6%). The mt genome had an overall high A+T content of 75.1% with content levels of 74.7% in PCGs, 77.5% in tRNA genes, 79.1% in rRNA genes, and 72.2% in the control region (Table 3). When performing skew statistics on the PCGs, it was clearly shown that PCGs on the J strand were TA-skewed, while the N strand was AT-skewed.

**Results**

**Features of the *L. pratensis* mt Genome**

The complete *L. pratensis* mt genome is 16,591 bp double-stranded circular molecule (Fig. 1), containing 37 genes (13 PCGs, 22 tRNAs, and 2 rRNAs) and a noncoding region (control region). Twenty-three of the genes were located on the majority strand (J strand, direction: F), while the remaining were on the minority strand (N strand, direction: R). In the mitogenome, genes were observed to be overlapped, contiguous, or separated by a few nucleotides. Gene overlaps were observed at 11 gene junctions, with the longest overlap (14 bp) present between the termination of ATPase6 and the initiation of COIII. There were seven spacer sequences in the *L. pratensis* mitogenome, with the longest intergenic spacer sequence (7 bp) located between tRNA^Ser^ and ND1. The entire *L. pratensis* mt genome sequence was deposited in GenBank under accession number MF188235.

**Protein-Coding Genes**

The PCGs (11,087 bp) comprised 66.83% of the whole mt genome. Thirteen of the genes were strictly initiated with an ATN start codon to include ATT (6), ATG (5), ATA (1), and ATC (1). The conventional stop codon TAN was also observed to include TAA (9), TAG (1). Furthermore, three of the PCGs (COI, COIII, and ND3) were terminated with a single T as the stop codon (Table 2).

**tRNA and rRNA Genes**

Within the *L. pratensis* mt genome, 22 tRNA genes were identified, with 21 identified using tRNAscan-SE and 1 by sequence comparison with other Hemipterans. All of the identified tRNAs had lengths between 62 bp (tRNA^Thr^, tRNA^Asp^, and tRNA^His^) and 70 bp (tRNA^Leu^; Table 2) and were predicted to have classic clover leaf structures. Furthermore, TWPC loops were found to be missing in tRNA^Aaw^, tRNA^His^, and tRNA^His^$. Additionally, 22 unmatched base pairs were predicted within 11 of the tRNAs, with 20 of them being located in the AA arm (9 bp), the TWPC stem (3 bp), or DHU stem (8 bp). The remaining 2 unmatched base pairs included a U-U mismatch in the AA stem of tRNA^Aaw^, and a U-C mismatch in the AA stem of tRNA^Aaw^ (Fig. 3).

In addition to the identified tRNAs, two rRNAs were also identified. The lrRNA gene (1,250 bp) was found to be located between the tRNA^Val^ and tRNA^Ser^ genes, while the srRNA gene (1,250 bp) was located between the tRNA^Val^ gene and the control region. The
AT content was 79.1% for these rRNA genes, with 1rRNA having a content level of 79.8% and 2rRNA a level of 78.2%.

Noncoding and Overlapping Regions

The mt noncoding region was found to include short intergenic spacers and a long control region. The *L. pratensis* mitogenome harbors a total of 17 intergenic spacer sequences that vary from 1 to 7 bp in length and are distributed in seven regions. It also contains a long control region (2,017 bp) that is located between the 12S-rRNA and tRNA-Ile-Gln-Met gene cluster. The control region was found to have an AT content of 72.19%, with a positive AT skew and negative GC skew noted. Moreover, the complete control region contains three tandem repeats and includes a short 24 bp sequence tandemly repeated four times; a 25 bp sequence tandemly repeated three times.

**Table 2.** Organization of the *Lygus pratensis* mt genome

| Gene       | Direction | Strand | Position (bp) | Anticodon | Size (bp) | Start codon | Stop codon | Intergenic nucleotide (bp) |
|------------|-----------|--------|---------------|-----------|-----------|-------------|------------|---------------------------|
| tRNA-Ile (I) | F        | J      | 1–65          | GAT       | 65        | –           | –          | 0                         |
| tRNA-Gln (Q) | R        | N      | 63–131        | TTG       | 69        | –           | –          | −3                        |
| tRNA-Met (M) | F        | J      | 132–199       | CAT       | 68        | –           | –          | 0                         |
| ND2        | F        | J      | 200–1,207     | –         | 1,008     | ATT         | TAA        | 0                         |
| tRNA-Trp (W) | F        | J      | 1,206–1,270   | TCA       | 65        | –           | –          | −2                        |
| tRNA-Cys (C) | R        | N      | 1,262–1,325   | GCA       | 63        | –           | −8         |
| tRNA-Tyr (Y) | R        | N      | 1,325–1,391   | GTA       | 66        | –           | –          | 0                         |
| COI        | F        | J      | 1,392–2,925   | –         | 1,534     | ATG         | TAA        | 0                         |
| tRNA-Leu (L) | F        | J      | 2,926–2,991   | TAA       | 66        | –           | –          | 0                         |
| COII       | F        | J      | 2,992–3,672   | –         | 681       | ATC         | TAA        | 0                         |
| tRNA-Lys (K) | F        | J      | 3,673–3,743   | CTT       | 70        | –           | –          | 0                         |
| tRNA-Asp (D) | F        | J      | 3,745–3,810   | GTC       | 65        | –           | –          | 2                         |
| ATPase8    | F        | J      | 3,809–3,971   | –         | 162       | ATT         | TAG        | 0                         |
| ATPase6    | F        | J      | 3,965–4,648   | –         | 684       | ATG         | TAA        | 0                         |
| COIII      | F        | J      | 4,634–5,418   | –         | 784       | ATG         | T          | −14                       |
| tRNA-Gly (G) | F        | J      | 5,419–5,481   | TCC       | 62        | –           | –          | 0                         |
| ND3        | F        | J      | 5,482–5,832   | –         | 352       | ATA         | T          | 0                         |
| tRNA-Ala (A) | F        | J      | 5,834–5,895   | TGC       | 62        | –           | −1         |
| tRNA-Arg (R) | F        | J      | 5,899–5,965   | TCG       | 67        | –           | 3          |
| tRNA-Asn (N) | F        | J      | 5,964–6,028   | GTT       | 65        | –           | −2         |
| tRNA-Ser (S) | F        | J      | 6,028–6,096   | GCT       | 69        | –           | −1         |
| tRNA-Glu (E) | F        | J      | 6,096–6,159   | TTC       | 64        | –           | −1         |
| tRNA-Phe (F) | R        | N      | 6,158–6,219   | GAA       | 62        | –           | −2         |
| ND5        | R        | N      | 6,220–7,918   | –         | 1699      | ATT         | TAA        | 0                         |
| tRNA-His (H) | R        | N      | 7,919–7,983   | GTG       | 64        | –           | −3         |
| ND4        | R        | N      | 7,984–9,311   | –         | 1,327     | ATG         | TAA        | 0                         |
| ND4L       | R        | N      | 9,303–9,608   | –         | 306       | ATT         | TAA        | −7                        |
| tRNA-Thr (T) | F        | J      | 9,611–9,678   | TGT       | 68        | –           | 2          |
| tRNA-Pro (P) | R        | N      | 9,679–9,742   | TGG       | 64        | –           | 0          |
| ND6        | F        | J      | 9,744–10,232  | –         | 489       | ATT         | TAA        | 1                         |
| CytB       | F        | J      | 10,234–11,367 | –         | 1134      | ATG         | TAA        | 1                         |
| tRNA-Ser (S) | F        | J      | 11,368–11,433 | TGA       | 67        | –           | −1         |
| ND1        | R        | N      | 11,441–12,367 | –         | 927       | ATT         | TAA        | 7                         |
| tRNA-Leu (L) | R        | N      | 12,368–12,431 | TAG       | 64        | –           | 0          |
| 16S-rRNA (lrRNA) | R    | N      | 12,432–13,681 | –         | 1250      | ATT         | TAA        | 0                         |
| tRNA-Val (V) | R        | N      | 13,682–13,750 | TAC       | 69        | –           | 0          |
| 12S-rRNA (srRNA) | R    | N      | 13,751–14,575 | –         | 824       | –           | 0          |
| Control region | –        | –      | 14,576–16,591 | –         | 2,017     | –           | –          | 0                         |

**Table 3.** *Lygus pratensis* mt genome nucleotide composition and skew

| Feature        | %A  | %C  | T%  | G%  | A+T% | AT skew | GC skew |
|----------------|-----|-----|-----|-----|------|---------|---------|
| Whole genome   | 42.7| 14.6| 32.4| 10.3| 75.1 | 0.14    | −0.17   |
| PCGs           | 43.8| 10.4| 30.9| 14.9| 74.7 | 0.17    | 0.18    |
| PCGs (J)       | 39.2| 15.2| 33.8| 11.8| 73.0 | 0.07    | −0.13   |
| PCGs (N)       | 43.6| 7.0 | 22.4| 12.5| 66.0 | 0.32    | 0.28    |
| tRNA genes     | 41.3| 10.1| 36.2| 12.4| 77.5 | 0.07    | 0.10    |
| tRNA genes (J) | 41.1| 10.8| 36.5| 11.6| 77.6 | 0.06    | 0.10    |
| tRNA genes (N) | 41.7| 15.2| 35.9| 7.3 | 77.6 | 0.07    | −0.35   |
| rRNA genes     | 44.2| 12.8| 34.9| 8.1 | 79.1 | 0.12    | −0.22   |
| Control region | 36.8| 16.2| 35.4| 11.7| 72.2 | 0.02    | −0.16   |

*AT skew = (A−T)/(A+T),
*GC skew = (G−C)/(G+C).*

AT content was 79.1% for these rRNA genes, with lrRNA having a content level of 79.8% and srRNA a level of 78.2%.

Noncoding and Overlapping Regions

The mt noncoding region was found to include short intergenic spacers and a long control region. The *L. pratensis* mitogenome harbors a total of 17 intergenic spacer sequences that vary from 1 to 7 bp in length and are distributed in seven regions. It also contains a long control region (2,017 bp) that is located between the 12S-rRNA and tRNA-Ile-Gln-Met gene cluster. The control region was found to have an AT content of 72.19%, with a positive AT skew and negative GC skew noted. Moreover, the complete control region contains three tandem repeats and includes a short 24 bp sequence tandemly repeated four times; a 25 bp sequence tandemly repeated three times,
with a partial forth; and a 61 bp sequence tandemly repeated eight times (Fig. 4). For the overlapping sequences, a total of 48 bp were identified, throughout 11 regions and ranged in size from 1 to 14 bp (Table 2).

Phylogenetic Analysis
Phylogenetic associations were examined among four Cimicomorpha families, with 14 mt genome sequences utilized. The NJ, ML, and BI analyses all basically generated the same tree topologies (Fig. 5). These findings showed that: 1) *L. pratensis* is clustered in the same clade as three of the *Lygus* species; 2) a sister relationship between *A. lucorum* and several *Lygus* species was highly supported; 3) among the 11 Miridae mtDNA sequences, 9 species formed a monophyletic; and 4) within Cimicomorpha, Miridae is a sister group to the Anthocoridae (*O. niger*), Reduviidae (*A. dohni*), and Nabidae (*G. annulatus*) families.

Discussion
In the present study, the complete *L. pratensis* mt genome (16,591 bp) was cloned, assembled, and analyzed. Similar to most insects, this mt genome contained the standard 13 PCGs, 22 tRNA genes, 2 rRNA genes, and a control region (Zhang et al. 2013). It also shares the same gene arrangement as most of the sequenced Heteroptera mt genomes (Song et al. 2016). Among the *Lygus* mitogenomes reported in GenBank, *L. hesperus* (17,747 bp) is the largest, while *L. rugulipennis* (15,819 bp) is the smallest. All of the identified PCGs have conventional start codons (ATN), and nine have conventional stop codons (TAN). However, three had stops with a single T, with this phenomenon of an incomplete termination codon found in some insects, and sometimes at high frequencies (Liao et al. 2010), and this is presumed to be generated by posttranscriptional polyadenylation (Lee et al. 2006, Cha et al. 2007).

The nucleotide composition showed an AT bias, similar to that seen in the metazoan mt genome, and it is similar to the common strand biases of other bug mtDNA. In the *L. pratensis* mitogenome, 13 PCGs showed higher TA and GC skews in the N strands than in the J strands, while the J strand tRNAs had a higher GC skew than the N strands. This observation is most likely attributed to asymmetrical mutational constraints during replication (Hassanin et al. 2005, Min and Hickey 2007). Furthermore, the nucleotide AT bias was also reflected in the high AT usage, within codons, with TTT (F), TTA (L), ATT (I), and ATA (I) being the most prevalent.

All of the tRNAs were predicted to form classic clover leaf structures. While the TVC stem, TVC loop, DHU stem, and DHU loop were often variable, the AA arms (7 bp), anticodon loops (7 bp), and anticodon stems (5 bp) were invariant. In other insects, some tRNAs have been reported to form DHU arms with one typical loop and deficient DHU stem (Li et al. 2007a, Song and Liang 2009), but herein, the *L. pratensis* DHU arms were predicted with complete DHU stems and loops. However, mismatched and aberrant loops do exist in some tRNAs (Song et al. 2013). As to whether the function of these abnormal tRNAs is affected, some researchers have proposed that a posttranscriptional RNA editing mechanism might maintain the function of these tRNA genes (Tomita et al. 2002).

Within the mt genome, rRNAs are highly conserved and evolve slowly. It is difficult to precisely determine the ends of rRNA genes, and herein, the ends were assumed to extend to the boundaries of adjacent genes (Boore 2006); however, for the srRNA gene, the 3′ end was determined by comparison with other reported Hemipteran mitogenomes. As is seen in other Miridae species (Wang et al. 2014a), the *L. pratensis* mitogenome was found to harbor two rRNA genes (lrRNA and srRNA), but further examination into their secondary structures is still required.

The noncoding region located between 12S and tRNA^Ile^ was annotated as the control region, which is involved in original replication and contains the presumed transcriptional initiation (Zhang et al. 1995). This is one of the most variable regions within the mt genome, and contains high rates of nucleotide substitution, insertion, and deletion, with some varied tandemly repeated elements also present at times. The control region is the largest noncoding region in the *L. pratensis* mt genome (2,017 bp), with the 5′ end adjoining the 12S rDNA and the 3′ end adjoining to tRNA^Ile^, The varying size of the control region is due to repeat unit copy number variations (Zhang and Hewitt 1997, Wang et al. 2014b). At present, intragenus variations in mtDNA control region, sequences appear to
be attributed to distinct structural and evolutionary characteristics, including variable size, conserved structural elements, and tandem repeat sequences. This has been observed in the 'Adelphocoris' and 'Lygus' genera within the Miridae (Wang et al. 2014a), as well as other true bugs in Hemiptera (Dai et al. 2012; Li et al. 2012, 2013; Song et al. 2013). It has also been suggested that the existence of tandem repeats might be due to replication slippage (Fumagalli et al. 1996). These findings suggest that the variable control region sizes and abundance of tandem repeat elements may be helpful for evolutionary and population genetics studies of this genus.

Phylogenetic analyses clearly revealed a close relationship between L. pratensis and three other Lygus bugs, and it was identified...
as a sister group to Apolygus and Adelphocoris. Moreover, Miridae was found to be a sister group to three other families within Cimicomorpha, but more taxonomic examination is still required. The findings presented herein are consistent with previous studies (Wang et al. 2014a, b), and contribute an additional mt genome sequence that can be utilized in Miridae genealogical classifications, and further shows why mtDNA is an ideal molecular marker for evolutionary and phylogenesis studies.

In conclusion, the present study confirmed the usability of mtDNA in a phylogenesis study of the Lygus genus, within Miridae.

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