Characterization of the complete mitochondrial genome of *Myrmus lateralis* (Heteroptera, Rhopalidae) and its implication for phylogenetic analyses

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Academic editor: Laurence Livermore | Received 12 August 2021 | Accepted 25 October 2021 | Published 10 November 2021

Abstract

Mitochondrial genomes (mitogenomes) are widely used in research studies on phylogenetic relationships and evolutionary history. Here, we sequenced and analyzed the mitogenome of the scentless plant bug *Myrmus lateralis* Hsiao, 1964 (Heteroptera, Rhopalidae). The complete 17,309 bp genome encoded 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and a control region. The mitogenome revealed a high A+T content (75.8%), a positive AT-skew (0.092), and a negative GC-skew (−0.165). All 13 PCGs were found to start with ATN codons, except for *cox1*, in which TTG was the start codon. The Ka/Ks ratios of 13 PCGs were all lower than 1, indicating that purifying selection evolved in these genes. All tRNAs could be folded into the typical cloverleaf secondary structure, except for *trnS1* and *trnV*, which lack dihydrouridine arms. Phylogenetic trees were constructed and analyzed based on the PCG+rRNA from 38 mitogenomes, using maximum likelihood and Bayesian inference methods, showed that *M. lateralis* and *Chorosoma macilentum* Stål, 1858 grouped together in the tribe Chorosomatini. In addition, Coreoidea and Pyrrhocoroidea were sister groups among the superfamilies of Trichophora, and Rhopalidae was a sister group to Alydidae + Coreidae.

Keywords

Mitogenome, next-generation sequencing, phylogeny, scentless plant bugs
Introduction

Mitochondria are important cytoplasmic organelles in eukaryotic cells, playing a critical role in cell metabolism, disease, apoptosis, and senescence. Mitochondrial genomes are characterized by a simple structure, stable composition, conserved arrangement, maternal inheritance, and rapid evolutionary rate (Simon et al. 2006; Cameron 2014; Tyagi et al. 2020). Therefore, mitochondrial genomes (mitogenomes) have been widely used in phylogenetic analyses and studies on population genetics (Yuan et al. 2015; Song et al. 2019; Xu et al. 2021). For most insects, the mitogenome is typically a double-stranded circular DNA molecule from 15 to 20 kb in size, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, as well as a long non-coding region, also known as the control or AT-rich region (Wolstenholme 1992; Boore 1999; da Fonseca et al. 2008; Yang et al. 2018). Next-generation sequencing enables large amount of sequence data to be obtained and analyzed in a more economically efficient manner. As a result of advances in this technology, it is now more feasible to obtain the complete mitogenome of large taxa; thus, molecular phylogenetic analysis has been revolutionized (Beckenbach et al. 2009; Kocher et al. 2014).

Rhopalidae, commonly known as scentless plant bugs, is a family of Pentatomomorpha insects in the superfamily Coreoidea (Schaefer and Chopra 1982; Schaefer and Panizzi 2000; Steill and Meyer 2003). The Rhopalidae includes 26 genera and 279 species distributed throughout the biogeographical areas of the world (CoreoideaSF Team 2021); many species are of critical importance as pests of pasture grass (Carroll and Loye 2012). In China, Rhopalidae includes two subfamilies, Serinethinae (containing only one genus) and Rhopalinae (containing 20 genera) (Dolling 2006). Phylogeny for the tribes of Rhopalinae has been hypothesized based on morphology, but several concurrent hypotheses still exist (Schaefer 1993; Li and Zheng 1994; Davidova-Vilimova et al. 2000; Henry 2019). Although there are several published studies examining the relationships among infrafamilial levels of the Coreoidea, a consensus regarding the relationships among different families and subfamily lineages has not been reached (Souza et al. 2009; Ghahari et al. 2012; Forthman et al. 2019). In addition, some studies support Aradoidea as sister group of Trichophora (which includes Pentatomoidea, Lygaeoidea, Pyrrhocoroidea and Coreoidea), but the relationship within Trichophora is still under debate (Li et al. 2005; Xie et al. 2005; Linnavuori 2007; Tian et al. 2011; Weirauch and Schuh 2011).

Myrmus lateralis Hsiao, 1964 is an endemic species of China (Beijing, Hebei, eastern Inner Mongolia), Korea, Mongolia, and Russia (Far East) (Liu et al. 1994; Aukema and Rieger 2006; CoreoideaSF Team 2021). Adults are present in both forests and grasslands during July and August (Nonnaizab 1986). To date, only four complete mitogenomes from the Rhopalidae have been published in GenBank, and all of them belong to Rhopalinae (Table 1). According to the latest classification, five tribes are classified in the Rhopalidae (CoreoideaSF Team 2021). The tribe Chorosomatini Fieber, 1860 contains five genera (Agraphopus Stål, 1872; Chorosoma Curtis, 1830; Ithamar Kirkaldy, 1902; Leptoceraea Jakovlev, 1873; Myrmus Hahn, 1832 and Xenogenus Berg, 1883), of which only Myrmus and Chorosoma are distributed in China.
Table 1. List of sequences used to reconstruct the phylogenetic tree.

| Family           | Species                  | Accession number |
|------------------|--------------------------|------------------|
| Outgroup         |                          |                  |
| Nabidae          | Himacerus nodipes        | JF927832         |
| Ingroup          |                          |                  |
| Aradidae         | Aradacanthius heissi     | HQ441233         |
|                  | Aradus compar            | NC_030362        |
|                  | Aneurus similis          | NC_030360        |
| Cydnidae         | Macroc dysus gibbulus    | NC_012457        |
|                  | Adria magna              | NC_042429        |
|                  | Scoparipes salvazai      | MF614955         |
| Pentatomidae     | Eurydema dominulus       | NC_044762        |
|                  | Palomena viridisima      | NC_050166        |
|                  | Euyparcoris guttigerus   | MN831205         |
|                  | Arma cuitos              | MT535604         |
|                  | Pentatoma semiannullata  | MT985377         |
| Colobiathristidae| Phaeacanthia marcida     | EU427342         |
| Rhyparochromidae | Panaurus albomaculatus   | NC_031364        |
| Malidae          | Malus inopinicus         | EU427339         |
| Lygaeidae        | Kleidocerys reseda       | KJ584365         |
| Pyrrhocoridae    | Dindymus rubiginosus      | NC_042439        |
|                  | Eucopas rufipes          | NC_042436        |
|                  | Melamphaus faber         | NC_042435        |
|                  | Dysdercus decusatus      | NC_042438        |
|                  | Antilaechus coquebertii  | NC_042441        |
| Rhopalidae       | Stictopleurus suboviridis| EU826088         |
| Myrmus lateralis |                          | MN412595         |
|                  | Chorormosa macilentum    | MN412594         |
|                  | Aeschyntelus notatus     | EU427333         |
|                  | Corizus sp.              | KM983397         |
| Alydidae         | Riptortus pedestris      | EU427344         |
| Coreidae         | Neomegalotomus parvus    | MG253274         |
|                  | Hydaranpis longirostris  | EU427337         |
|                  | Clavigalla tomentosicollis| KY274846        |
|                  | Acansibocoris sp.        | MF497707         |
|                  | Enoptopus potanini       | MF497720         |
|                  | Leptoglossus membranaceus| MF497724         |
|                  | Anoplocnemis curvipes    | KY906999         |
|                  | Micris tenebrosa         | MF497729         |
|                  | Pseudococis brevicornis  | MF497732         |
|                  | Molipteryx lunata        | MF497721         |
|                  | Notopteryx soror         | KX505857         |

In this study, we sequenced and annotated the complete mitogenome of *M. lateralis*. We examined the genomic features, nucleotide composition, codon usage, RNA secondary structure, evolutionary pattern of 13 PCGs and characteristics of the control region. Finally, we evaluated the phylogenetic relationship of the mitochondrial sequence data at different taxonomic levels.

**Material and methods**

**Sampling, DNA extraction and sequencing**

Adult specimens of *Myrmus lateralis* were collected from Jincheng City, Shanxi Province, China on 20 July 2014. Specimens were preserved in 100% ethanol and stored at −20 °C. The genomic DNA was extracted from leg muscles using a ONE-4-ALL
Genomic DNA Mini-Prep Kit (BS88504; Sangon, Shanghai, China) according to the manufacturer’s protocols. The mitogenome was sequenced using the whole-genome shotgun method on an Illumina Miseq platform (Personalbio, Shanghai, China). After filtering low-quality and adapter contaminated reads, A5-miseq version 20150522 (Coil et al. 2015) was used for contig assembly.

Gene annotation and sequence analysis

Sequence annotation was performed using Geneious 10.1.3 (Kearse et al. 2012) and the MITOS web server (Bernt et al. 2013). Annotations of 13 protein-coding regions were edited manually by predicting open reading frames using the invertebrate mitochondrial code. The secondary structures of the tRNA genes were predicted with tRNAscan-SE web server (http://lowelab.ucsc.edu/tRNAscan-SE/) (Tamura et al. 2013). The identification of rRNA genes was performed based on their putative secondary structures and by comparing nucleotide sequences with those of previously reported mitogenomes. The control region was identified through the boundary of neighboring genes. The number of tandem repeats of control region was investigated with Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.html) (Benson 1999) and the presence of stem-loop was predicted by Mfold Web Server (http://mfold.rna.albany.edu) (Zuker 2003). The annotated mitogenome sequence of *Myrmus lateralis* has been submitted to GenBank under the accession number MN412595.

The base compositions, codon usages, and relative synonymous codon usage (RSCU) values were calculated by MEGA 6.0. AT and GC skew were calculated by the following formulae: AT skew = (A−T)/(A+T) and GC skew=(G−C)/(G+C). To analyse the evolutionary patterns of the 13 PCGs, the rates of nonsynonymous substitution (Ka), the rates of synonymous substitution (Ks), and the ratio of Ka/Ks for each gene were calculated by the software DnaSP 5.0 (Librado and Rozas 2009).

Phylogenetic analyses

In addition to the mitogenome newly sequenced here, 37 other mitogenomes were taken from Genbank for the phylogenetic analyses (Table 1). The nucleic acids of 13 PCGs and two rRNAs were extracted by Geneious 10.1.3 and aligned with the Muscle algorithm in MEGA 6.0. Finally, the individual alignments were concatenated to make the datasets of PCG+rRNA (nucleotide alignment including 13 protein-coding genes and two rRNA genes) with SequenceMatrix (Vaidya et al. 2011).

The dataset was used to reconstruct phylogenetic trees under Bayesian inference (BI) and maximum likelihood (ML) using MrBayes 3.2.6 (Ronquist et al. 2012) and RaxML 8.0.2 (Stamatakis 2015) respectively. BI analysis was performed using a TVM+I+G model selected by jModeltest (Darriba et al. 2012). Two independent runs of four Markov Chains Monte Carlo (MCMC) chains in parallel for 5,000,000 generations were applied and sampled every 1,000 generations. Tracer 1.7 (Rambaut et al. 2018) was used to analyze the trace files from the Bayesian MCMC runs. Stationarity was
considered to be reached when ESS (estimated sample size) value above 100 as MrBayes suggested. The first 25% of sampled trees were discarded as burn-in, and the remaining trees were used to calculate a 50% majority rule consensus tree. The ML tree was constructed using the GTR+I+G model, and nodal support values were evaluated through an ultrafast bootstrap approach, with 10,000 replicates.

**Results**

**Genome organization**

The 17,309 bp mitochondrial genome of *Myrmus lateralis* was determined to be a typical circular nucleotide molecule, consisting of 13 PCGs, 22 tRNA genes, two rRNA genes, and a putative control region (Table 2). For the gene arrangement, 23 genes (nine PCGs and 14 tRNAs) were found on the majority strand and 14 genes (four PCGs, eight tRNAs, and two rRNAs) were found on the minority strand. In addition, the mitogenome contains 10 gene overlaps totaling 37 bp and ranging from 1 to 8 bp; the longest overlap is being found between *trnW* and *trnC*. Thirteen intergenic spacers equal to 77 bp were observed, ranging from 1 to 18 bp, the longest being found between *trnS2* and *nad1*.

**Nucleotide composition and codon usage**

The mitochondrial genome of *M. lateralis* was strongly biased toward A+T (75.8%) in nucleotide composition, with 75.5%, 75.7%, 71.3%, and 74.8% A+T content in PCGs, rRNAs, tRNAs, and control regions, respectively. The nucleotide composition and skewness of the mitogenome is shown in Table 3. The three codon positions of PCGs possessed different A+T content; the third site had the highest value (80.1%), whereas the second site had the lowest (74.3%). The mitogenome had more A and G content than T and C, with a positive AT-skew (0.092) and a negative GC-skew (−0.165). The AT-skew of PCGs and three codon positions were all negative, −0.018 in PCGs, −0.214 in the first, −0.050 in the second, and −0.099 in the third codon positions. The GC-skew of PCGs (0.033) and the second codon position (0.127) was slightly and moderately positive, respectfully, while the first and third codon positions were both negative (−0.018 and −0.020, respectively). AT-skew values for the tRNA and rRNA genes were both negative (−0.057 and −0.001, respectively), while the GC-skew was obviously positive for tRNAs (0.292) and slightly positive for rRNAs (0.019).

Relative synonymous codon usage (RSCU) values of the 13 PCGs were calculated based on 3633 codons (Fig. 1). UUU (F, Phe), UAU (Y, Tyr), UUA (L, Leu2), and AUU (I, Ile) were the most frequently used codons, accounting for 28.24% of all codons. All synonymous codons ending with A or U were more frequent than those ending with C or G, except in three cases: CGG (RSCU = 0.97) was used more than CGU (RSCU = 0.55) for Arg, AGG (RSCU=1.03) was used more than AGA (RSCU = 0.99) for Ser1, and GGG (RSCU=1.27) was used more than GGU (RSCU = 1.12) for Gly.
### Table 2. Organization of the mitochondrial genome of *Myrmus lateralis*.

| Gene     | Strand | Size(bp) | Start | Stop  | Intergenic nucleotides (IGN) | Anticodon | Codons |
|----------|--------|----------|-------|-------|------------------------------|-----------|--------|
| trnI     | J      | 66       | 1     | 66    | 0                            | GAU       | —      |
| trnQ     | N      | 69       | 64    | 132   | 2                            | UUG       | —      |
| trnM     | J      | 69       | 137   | 205   | 4                            | CAU       | —      |
| nad2     | J      | 985      | 207   | 1191  | 1                            | ATG       | T      |
| trnW     | J      | 63       | 1204  | 1266  | 12                           | UCA       | —      |
| trnC     | N      | 62       | 1259  | 1320  | —8                           | GCA       | —      |
| trnY     | N      | 64       | 1321  | 1384  | 0                            | GUA       | —      |
| cox1     | J      | 1539     | 1387  | 2925  |                              | —         |        |
| trnW     | J      | 66       | 2921  | 2986  | -5                           | UAA       | —      |
| cox2     | J      | 676      | 2987  | 3662  | 0                            | ATT       | T      |
| trnK     | J      | 72       | 3663  | 3734  | 0                            | CUU       | —      |
| trnD     | J      | 65       | 3738  | 3802  | 3                            | GUC       | —      |
| atp8     | J      | 162      | 3803  | 3964  | 0                            | —         | ATA    |
| atp6     | J      | 669      | 3958  | 4626  | -7                           | —         | ATG    |
| cox3     | J      | 790      | 4626  | 5415  | -1                           | —         | ATG    |
| trnG     | J      | 63       | 5413  | 5475  | -3                           | UCC       | —      |
| nad3     | J      | 349      | 5476  | 5824  | 0                            |          |        |
| trnA     | J      | 63       | 5825  | 5887  | 9                            | UGC       | —      |
| trnK     | J      | 64       | 5891  | 5954  | 3                            | UCG       | —      |
| trnN     | J      | 66       | 5954  | 6019  | -1                           | GUU       | —      |
| trnS1    | J      | 69       | 6023  | 6091  | 3                            | GCU       | —      |
| trnE     | J      | 64       | 6091  | 6154  | -1                           | UUC       | —      |
| trnF     | N      | 66       | 6154  | 6219  | -1                           | GAA       | —      |
| nad5     | N      | 1702     | 6220  | 7921  | 0                            | —         | ATA    |
| trnH     | N      | 63       | 7931  | 7993  | 9                            | GUG       | —      |
| nad4     | N      | 1317     | 7994  | 9310  | 0                            | —         |        |
| nad4L    | N      | 288      | 9304  | 9591  | -7                           | —         |        |
| trnT     | J      | 62       | 9594  | 9655  | 3                            | UGU       | —      |
| trnP     | N      | 64       | 9678  | 9719  | 0                            | UGG       | —      |
| nad6     | J      | 483      | 9728  | 10210 | 8                            | —         | ATA    |
| cytB     | J      | 1137     | 10210 | 11346 | -1                           | —         | ATG    |
| trnS2     | J      | 69       | 11345 | 11413 | -2                           | UGA       | —      |
| nad1     | N      | 927      | 11432 | 12358 | 18                           | —         |        |
| trnL2    | J      | 67       | 12359 | 12425 | 0                            | UAG       | —      |
| trnL     | N      | 1261     | 12426 | 13686 | 0                            | —         | —      |
| trnV     | N      | 69       | 13687 | 13755 | 0                            | UAC       | —      |
| trnS     | N      | 967      | 13756 | 14722 | 0                            | —         | —      |
| Control region | 2587 | 14723 | 17309 | 0    | —                            | —         | —      |

Note: J refers to major strand; N refers to minus strand

### Table 3. Nucleotide composition and skewness of the *Myrmus lateralis* mitochondrial genome.

| Feature          | T%  | C%  | A%  | G%  | A+T% | AT-skew | GC-skew |
|------------------|-----|-----|-----|-----|------|---------|---------|
| Whole genome     | 34.4| 14.1| 41.4| 10.1| 75.8 | 0.092   | -0.165  |
| PCGs             | 42.2| 11.8| 33.3| 12.6| 75.5 | -0.118  | 0.033   |
| PCGs-1<sup>st</sup> | 44  | 14.1| 28.5| 13.6| 72.5 | -0.214  | -0.018  |
| PCGs-2<sup>nd</sup> | 39  | 11  | 35.3| 14.2| 74.3 | -0.05   | 0.127   |
| PCGs-3<sup>rd</sup> | 44  | 10.4| 36.1| 10  | 80.1 | -0.099  | -0.02   |
| tRNAs           | 40  | 8.6 | 35.7| 15.7| 75.7 | -0.057  | 0.292   |
| rRNAs           | 35.7| 14.1| 35.6| 14.7| 71.3 | -0.001  | 0.019   |
| Control region  | 35  | 15.9| 39.8| 9.4 | 74.8 | 0.064   | -0.257  |
Figure 1. The relative synonymous codon usage (RSCU) in the *Myrmus lateralis* mitogenome.

**Protein coding genes**

In the *M. lateralis* mitogenome, nine PCGs were located on the J-strand (majority strand) and four PCGs were located on the N-strand (minority strand). The PCGs had a total length of 11,024 bp that accounted for 63.69% of the complete mitogenome. Among the mitochondrial proteins, Leu (16.67%), Phe (11.02%), and Tyr (9.58%) were the most frequent amino acids.

Most PCGs started with a typical ATN codon; five started with ATG (*nad2, atp6, cox3, nad4*, and *cytb*), four with ATA (*atp8, nad3, nad5*, and *nad6*), and three with ATT (*cox2, nad4L*, and *nad1*). The only unusual initiation codon was TTG in *cox1*. Among the 13 PCGs, seven genes ended with the complete stop codon TAA (*cox1, atp8, atp6, nad1, nad4*, and *nad4L*) or TAG (*cytb*), whereas five genes ended with the partial termination codon T (*nad2, cox2, cox3, nad3*, and *nad5*).

The evolutionary patterns of the 13 PCGs were analyzed and shown in Figure 2. The highest values for both Ka and Ks were observed for *atp8* and *cytb*. *atp8* exhibited the highest Ka/Ks value, whereas *cox1* exhibited the lowest. The Ka/Ks values for all 13 PCGs were below 0.29.
Figure 2. The rates of nonsynonymous substitution (Ka), the rates of synonymous substitution (Ks), and the ratio of Ka/Ks for each PCGs of *Myrmus lateralis* mitogenome

Transfer RNAs and ribosomal RNAs

A total of 22 tRNA genes were encoded by the *M. lateralis* mitogenome, ranging from 62 bp (trnC and trnT) to 72 bp (trnK) in length (Table 2). Eight tRNA genes (trnQ, trnC, trnY, trnF, trnP, trnL1(CUN) and trnV) were encoded on the N-strand; the remaining 14 genes were encoded on the J-strand. The 22 tRNA genes had a total length of 1,445 bp, accounting for 8.34% of the complete mitogenome. The predicted secondary structures are shown in Figure 3. All tRNA genes could be folded into typical cloverleaf secondary structures, except for trnSI and trnV, in which the necessary dihydrouridine (DHU) arms were replaced with a simple loop. A total of 16 wobbled G-U pairs were found (six in acceptor stems, eight in DHU stems, one anticodon stems, and one in TΨC stems) that formed weak bonds in the tRNAs.

The large 1,261 bp *rrnL* gene was located between *trnL1(CUN)* and *trnV*, and the small 967 bp *rrnS* gene was located between *trnV* and the control region. The secondary structures of both the *rrnL* and *rrnS* genes are shown in Figures 4, 5. The secondary structure of the *rrnL* gene contained five domains (I, II, IV, V, VI); domain III was absent, whereas the *rrnS* gene consisted of three domains (I, II, III). Both rRNAs included many mismatched base pairs, most of which were G-U.
Figure 3. Predicted secondary structure of tRNA genes in the Myrmus lateralis mitogenome.
Figure 4. Predicted secondary structure of the *rrnL* in the *Myrmus lateralis* mitogenome.

Figure 5. Predicted secondary structure of the *rrnS* in the *Myrmus lateralis* mitogenome.
Control region

The 2587 bp mitochondrial control region of *M. lateralis* was located between the *rrnS* gene and *trnl*. The A+T content (74.76%) was higher than the G+C content (25.24%), with a positive AT-skew and a negative GC-skew. In the control region, tandem repeat sequences, polyT stretch, polyA stretch, stem-loop structure, tandem repeats, and G(A)\textsubscript{n} motif were commonly found. Based on these features, we identified the following important elements: a 13 bp polyT stretch, a G(A)\textsubscript{9}T sequence, an AT-AGA motif, and a 9 bp polyA stretch. In addition, a stem-loop structure was observed at the end of the control region (Fig. 6). Although no longer tandem repeat sequences were identified, stretches of T(A)\textsubscript{4} occurred many times.

Phylogenetic analyses

We conducted phylogenetic analyses based on the nucleotide sequences of the PCG+rRNA from eleven families within five superfamilies; one species from the Nabiidae (damsel bugs) was used as an outgroup. The dataset contained 14,128 nucleotide sites from these 38 taxa. The BI and ML analyses generated identical phylogenetic results with most posterior probabilities (PP) of one and bootstrap pseudoreplicates (BP) of 100 (Fig. 7). Pentatomoidea, Lygaeoidea, Pyrrhocoroidea, and Coreoidea, the trichophorans, were all monophyletic and highly supported in both analyses (PP > 0.85 and BP > 92). A sister relationship between Coreoidea and Pyrrhocoroidea was recovered, and Pentatomoidea formed a sister group with the other superfamilies of the remaining Trichophora. Both analyses provided robust support (PP > 0.95 and BP > 85) for three families within Coreoidea: Alydidae was closer to Coreidae than Rhopalidae. Sub-

![Figure 6. The stem-loop of control region in the *Myrmus lateralis* mitogenome.](image)
families of Coreidae were also supported with an internal relationship of ((Hydarinae + Pseudophloeinae) + Coreinae) (PP > 0.77 and BP > 75). Among Rhopalinae species, *Stictopleurus subviridis* Hsiao, 1977 was identified as sister to a group of other Rhopali- nae species (PP = 0.99 and BP = 100). *M. lateralis* was close to *Chorosoma macilentum* Stål, 1858 (PP = 1 and BP = 100) and they both belonged to the tribe Chorosomatini.

**Discussion**

In this study, we sequenced the mitogenome of *Myrmus lateralis* and built the molecular phylogenetic relationships with 37 other heteropteran taxa. The mitogenome was found to be 17,309 bp long, with 37 genes arranged consistent with other species of Hemiptera (Song et al. 2012; Li et al. 2017; Zhao et al. 2021). The control region represented the largest reported non-coding region for insect mitogenomes owing to its various motifs and tandem repeats (Cook et al. 1999; Li et al. 2015). Although the 2587 bp long control region possessed several elements, such as a polyT stretch, a G(A)_n T sequence, an ATAGA motif, a stem-loop, and a polyA stretch, tandem repeats were not observed.
The present study analyzed the initiation and termination codons of the 13 PCGs. The majority of PCGs used common start codons (ATN), except for *cox1*, which was initiated with TTG, a frequently used start codon in heteropteran mitogenomes (Quek et al. 2021; Zhao et al. 2021). The partial termination codon T was found in *nad2*, *cox2*, *cox3*, *nad3* and *nad5*. Some researchers have proposed that the incomplete termination codon could be completed via post-transcriptional polyadenylation (Ojala et al. 1981). Among the synonymous codons, codons ending with an A or U were more frequent than those ending in a G or C and were also observed in other heteropteran species (Zhao et al. 2019; Li et al. 2021). In this study, the lowest evolutionary rate was observed in *cox1*, indicating that this gene can be effectively used as a DNA barcoding marker. The Ka/Ks values for all 13 PCGs were far less than one, indicating that all of the PCGs evolved under purifying selection, thus, they can be used to investigate phylogenetic relationships.

Although many studies have reconstructed phylogenetic relationships among members of Pentatomomorpha, the relationships of different levels are still ambiguous (Li et al. 2005; Liu et al. 2019; Souza-Firmino et al. 2020; Kaur and Singh 2021). The results from previous research were congruent with the result that Trichophora includes four superfamilies of Pentatomomorpha except Aradoidea, and with the monophyly of four superfamilies of Trichophora (Henry 1997; Hua et al. 2008; Yuan et al. 2015; Song et al. 2019). Hua et al. (2008) and Liu et al. (2019) supported Pentatomoidea as the basal group of Trichophora and Coreoidea as a sister group to Lygaeoidea based on mitochondrial genomes. The superfamilies Coreoidea and Pyrrhocoroidea were supported as sister taxa based on cladistic analysis (Henry 1997), as well as 37 mitochondrial genes analysis (Yuan et al. 2015). Whereas our mitogenomic data showed that Coreoidea and Pyrrhocoroidea were sister groups based on ML and BI analyses using the PCG+rRNA dataset. This result from our study was consistent with that from the study of Song et al. (2019) based on relationship analysis of Pentatomomorpha PCGs.

At the family level, Rhopalidae was found to be a sister group to Alydidae + Coreidae. This relationship, based on mitogenomes, was consistent with that based on cladistic analysis by Li (1996) and Henry (1997). Despite the availability of numerous species, the classification patterns for the family Coreidae were weak in terms of both morphology and molecular data (Bressa et al. 2008; Forthman et al. 2019; Froeschner 2019). In this study, Hydaraeinae and Pseudophloeinae grouped the sister cluster. However, due to the limited samples, the relationships among Coreidae needs more studies in the future.

Previous studies on the classification of Rhopalinae came to a variety of conclusions and the positions of some species were not clear (Schaefer and Chopra 1982; Liu 1994; Aukema and Rieger 2006). The hypothesis that the genus *Stictopleurus* belongs to the Rhopalini tribe was corroborated based on morphological studies by Aukema and Rieger (2006). However, it did not group together with other Rhopalini species in this study. The monophyly of Rhopalini should be explored in the future studies with additional specimens.
Conclusions

The complete mitogenome of the scentless plant bug *Myrmus lateralis* was sequenced using next-generation sequencing technologies, providing the fifth mitogenome sequence from approximately 230 species of Rhopalidae. The nucleotide composition, codon usage, RNA structures, and protein-coding genes evolution were analyzed in our paper. The mitogenome of *M. lateralis* revealed the phylogenetic position of *Myrmus*. However, more mitogenomes should be sequenced to investigate the mitogenomic evolution and phylogenetic relationships of Rhopalidae.

Acknowledgments

This project was supported by the National Science Foundation Project of China (no. 31501876, 31872272) and supported by Scientific Research Project of Xinzhou Teachers University (2018KY04).

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