The Complete Mitochondrial Genome of *Ugyops* sp. (Hemiptera: Delphacidae)

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Received 11 February 2018; Editorial decision 31 May 2018

Abstract

The complete mitochondrial genome (mitogenome) of *Ugyops* sp. (Hemiptera: Delphacidae) was sequenced, making it the first determined mitogenome from the subfamily Asiracinae, the basal clade of the family Delphacidae. The mitogenome was 15,259 bp in length with A + T content of 77.65% and contained 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and a control region. The gene order was identical with that of the ancestral insect. The nucleotide composition analysis indicated that the whole mitogenome was strongly A-skewed (0.288) and highly C-skewed (−0.270). For PCGs on the J-strand, the AT skew was positive, and the GC skew was negative. All PCGs started with canonical ATN codons, except for *cox1* and *nad5*, which used CTG and GTG as start codons, respectively. All tRNAs could fold into typical cloverleaf secondary structures, with the exception of *trnS1* (*AGN*), in which the dihydrouridine arm was reduced to a simple loop. The control region included a poly-T stretch downstream of the small RNA gene (*rrnS*), a subregion of higher A + T content and tandemly repeated sequence near *rrnL*. The mitogenome of *Ugyops* sp. could be very helpful in exploring the diversity and evolution of mitogenomes in Delphacidae.

Key words: *Ugyops* sp., mitochondrial genome, gene arrangement, control region

The insect mitochondrial genome (mitogenome) generally encodes 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, and two ribosomal RNA (rRNA) genes (Boore 1999). These genes are typically arranged on a compact circular genome in the range of 15–18 kb (Cameron 2014a). In addition, there are some noncoding elements, with the largest one termed the control region regulating the transcription and replication of the mitogenome (Clayton 1982, 1992; Taanman 1999). The control region, alternatively called the A + T-rich region, is characterized by high A + T content and the occurrence of tandem repeat units (Zhang and Hewitt 1997).

The prevalent use of insect mitogenomes is phylogenetic analysis. Mitochondrial phylogenetics studies on the Hemiptera are extensive. The suborder Heteroptera has the largest number of published complete mitogenomes in Hemiptera (Song et al. 2016). Mitogenome sequencing is of much smaller scale within the suborder Auchenorrhyncha, especially within the infraorder Fulgoromorpha. Currently, only 11 complete mitogenomes have been sequenced in the superfamily Fulgoroidea (= Fulgoromorpha) (Hua et al. 2009; Song and Liang 2009; Song et al. 2010, 2012; Zhang et al. 2013, 2014, 2016a; Huang and Qin 2018a,b), including five species of Delphacidae: *Changodealpex velichkovskyi*, *Lasdelphax stratiellus*, *Nilaparvata lugens*, *Peregrinus maidis*, and *Sogatella furcifera*. Moreover, gene rearrangements are known for these species, with two clusters *trnW-trnC-trnY* and *trnI-trnP-nad6* undertaking conversion to *trnC-trnW-trnY* and *nad6-trnP-trnT*, respectively (Zhang et al. 2013, 2014).

The family Delphacidae is the most diverse and cosmopolitan group of the superfamily Fulgoroidea, with approximately 2,100 described species, of which the vast majority (80%) belong to the most species-rich subfamily Delphacinae (Urban et al. 2010, Huang et al. 2017). Species of Delphacidae feed on the phloem tissues of host plants, and a variety of species are economically significant pests of many important crops, such as rice and maize. Delphacid feeding causes serious yield losses of crops directly, but they are also vectors of phytoplasm, or viral plant pathogens (Wilson 2005). Approximately 30 delphacid species transmit plant viruses (Wilson 2005, Hogenhout et al. 2008).

The *Ugyops* Guérin-Méneville is an Oriental delphacid genus with 101 known species and is placed in the tribe Ugyopini of the subfamily Asiracinae (Fennah 1979, Bourgoin 2018). Phylogenetic analysis has shown that Asiracinae is not monophyletic and Ugyopini represents the earliest lineage in Delphacidae (Asche 1985, 1990; Emeljanov 1996). Comprehensive phylogenetic reconstruction of Delphacidae, combining nucleotide sequence and morphological characters, also indicated that Ugyopini (represented by two species...
of the genus *Ugyops*) was one of the most basal groups (Urban et al. 2010). The number of complete or nearly complete mitogenomes is slightly increasing in Auchenorrhyncha. However, relatively little is known about the mitogenomes from the tribe Ugyopini or the subfamily Asiracinae. In the present study, the complete mitogenome of *Ugyops* sp. was sequenced. This is the first representative mitogenome reported in the subfamily Asiracinae. Nucleotide composition, gene order, and other features were compared between *Ugyops* sp. and five species from Delphacinae mentioned above. Results from this work will facilitate the reconstruction of higher level phylogenetic relationships within Delphacidae and Fulgoroidea based on mitogenomic data in the future.

**Materials and Methods**

**DNA Extraction, Amplification, and Sequencing**

Adults of *Ugyops* sp. were collected in Sabah, Malaysia (5.443107°N, 116.451572°E). Samples were preserved in 100% ethanol and kept at −70°C until DNA extractions were conducted. The sequenced sample was deposited as voucher specimen in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s protocols. The mitochondrial genome of the *Ugyops* sp. was amplified using 11 pairs of primers (Supp Table 1 [online only]), which were modified from universal insect mitochondrial primers (Simon et al. 1994, Simon et al. 2006). All PCRs were performed in 50 μl reaction volumes using TaKaRa LA Taq (TaKaRa Biomedical, Dalian, China). The PCR thermal program was as follows: initial denaturation of 2 min at 94°C, followed by 35 cycles of 94°C for 1 min, 48–50°C for 1 min, 68°C for 10 min, and a final extension for 20 min at 68°C. The PCR products were electrophoresed in 1.2% agarose gel and sequencing was performed using BigDye v3.1 on an ABI 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, CA). When purified PCR products were difficult to sequence directly, they were inserted into a pMD 19-T Vector (TaKaRa Biomedical, Dalian, China). Multiple clones were independently sequenced.

**Annotation and Genomic Analysis**

The secondary structures of all tRNA genes were predicted using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) under the MITOS Web Server in W-IQ-TREE (Trifinopoulos et al. 2016, http://iqtree.lse. ac.uk). Secondary structures of two rRNAs (rrnL and rrnS) were inferred using models predicted for *Drosophila* spp. (Cannone et al. 2002), *Apis mellifera* (Gillespie et al. 2006), *Stenopirates* sp. (Li et al. 2012), *Cervaphis quercus* (Wang et al. 2014), *Panaurus albomaculatus* (Li et al. 2016), and *Taharana fasciana* (Wang et al. 2017). Helix names followed the conventions of Gillespie et al. (2006).

Nucleotide composition was calculated in Bioedit (Hall 1999). To measure the base-compositional difference, AT skew and GC skew were calculated using the formulae AT skew = (A − T)/(A + T) and GC skew = (G − C)/(G + C) (Perna and Kocher 1995). Codon usage and the relative synonymous codon usage (RSCU) were calculated with MEGA 6.0 (Tamura et al. 2013). The software DnaSP 5.0 (Librado and Rozas 2009) was used to calculate the number of synonymous substitutions per synonymous site (*Ks*), the number of nonsynonymous substitutions per nonsynonymous site (*Ka*), and the ratio of *Ka*/*Ks* for each PCG. The repeat motifs in the control region were detected using Tandem Repeats Finder (Benson 1999). Comparison of nucleotide composition, evolutionary rate, and noncoding region used the following five complete mitogenomes of Delphacidae from GenBank: *C. velitchkovskyi* (MG049916), *L. striatellus* (JX880068), *N. lugens* (NC_021748), *P. maidis* (MG049917), and *S. furcifera* (NC_021417).

**Sequence Alignment and Phylogenetic Analyses**

In total, 15 species were used for phylogenetic analyses, including eight species of Delphacidae and seven outgroup taxa (Table 1). Nucleotide sequence of each PCG was aligned individually based on alignment of translated amino acid sequence using Muscle (Edgar 2004) implemented in MEGA 6 (Tamura et al. 2013). All alignments were checked manually and then assembled into the concatenated data set. For the maximum likelihood (ML) and Bayesian inference (BI) analyses, the optimal partitioning schemes and best-fitting models (Supp Table 2 [online only]) were selected using PartitionFinder 2.1.1 (Lanfear et al. 2017) with the greedy algorithm under the corrected Akaike Information Criterion (AICc).

An ML tree was estimated using the IQ-TREE (Nguyen et al. 2015) Web Server in W-IQ-TREE (Trifinopoulos et al. 2016, http://iqtreeserver.com).

**Table 1. List of species used for phylogenetic analyses in this study**

| Superfamily | Family | Species | Accession number |
|-------------|--------|---------|-----------------|
| Ingroup     | Fulgoroidea | Delphacidae | Changeodelphax velitchkovskyi (MG049916), Lodelphax striatellus (JX880068), Nilaparvata bakhieri (NC_033388), Nilaparvata lugens (NC_021748), Nilaparvata muiri (NC_024627), Peregrinus maidis (MG049917), Sogatella furcifera (NC_021417), Ugyops sp. (MH352481) |
| Outgroup    | Fulgoroidea | Cixiidae | Pentastiridius sp. (KY039133), Fulgoridae | Lycorma delicatula (NC_012835), Issidae | Sivaloka damnosus (NC_014286), Riciandiidae | Ricana speculata (NC_031369), Aphrophoridae | Philaenus spumarius (NC_009494), Cercopidae | Abidama producta (NC_015799), Callitettix braconoides (NC_025497) |
with 1,000 replicates of ultrafast likelihood bootstrap (Minh et al. 2013). Bayesian trees were inferred using MrBayes V3.2.6 (Ronquist et al. 2012). Two Markov chain Monte Carlo (MCMC) runs were employed for 4,000,000 generations and trees were sampled every 500 generations. The 50% majority consensus tree was computed after excluding the first 25% of samples as burn-in.

Results and Discussion

Genome Organization

The mitochondrial genome of *Ugyops* sp. was 15,259 bp in length (GenBank MH352481), which is the smallest completely sequenced mitogenome in Fulgoroidea at present. The mitogenome contains 37 genes (13 PCGs, 22 tRNA genes, and two rRNA genes) and a control region, as found in most insects (Boore 1999) (Table 2).

The gene order of the *Ugyops* sp. mitogenome (Fig. 1) was identical to that of *Drosophila yakuba*, in which gene arrangement has been considered to be the ancestral gene order of insects (Clary and Wolstenholme 1985, Boore 1999). In Hemiptera, most species maintain the ancestral mitogenome arrangement of insects (Song et al. 2012, Cui et al. 2013, Wang et al. 2013, Liu et al. 2014, Li et al. 2016). Gene rearrangement, however, has been found in Aleyrodidae (Sternorrhyncha) (Thao et al. 2004), Cicadellidae (Auchenorrhyncha) (Du et al. 2017), Delphacidae (Auchenorrhyncha), and five families of true bugs (Heteroptera) (Hua et al. 2008, Li et al. 2012, Jiang et al. 2016, Song et al. 2016). Mitochondrial gene order changes, as one type of genomic changes, provide complementary markers with considerable potential for molecular systematics (Rokas and Holland 2000). In most insect orders, the synapomorphic rearrangements occur at many different taxonomic levels (Cameron 2014a).

The rearrangement was observed in species of the derived subfamily Delphacinae, and the gene order remained unknown in other subfamilies such as Vizcayinae, Pleisiodelphacinae, Kelisiinae, and Stenocraninae. Consequently, to explicate the origin and evolution of gene rearrangement, more delphacid mitogenomes are needed, particularly species from non-Delphacinae.

Nucleotide Composition

Results of comparative nucleotide composition of six delphacid species are listed in Table 3. The A + T content of *Ugyops* sp. mitogenome was 77.65%, and the nucleotide composition of the whole mitogenome was strongly A-skewed (0.288) and highly C-skewed (−0.270). Comparatively, a slightly A-skewed pattern was observed in five species of Delphacinae (Table 3).

### Table 2. Mitochondrial genome organization of *Ugyops* sp.

| Gene    | Strand | Position | Size (bp) | Anticodon | Start codon | Stop codon | Intergenic nucleotides (bp) |
|---------|--------|----------|-----------|-----------|-------------|------------|-----------------------------|
| trnI    | J      | 1–64     | 64        | GAT       | –           | –          | –                           |
| trnQ    | N      | 63–131   | 67        | TTT       | –           | –          | 0                           |
| trnM    | J      | 130–193  | 64        | CAT       | –           | –          | −2                          |
| nad2    | J      | 194–1153 | 960       | –         | ATT         | TAA        | 0                           |
| trnW    | J      | 1157–1219| 63        | TCA       | –           | –          | 3                           |
| trnC    | N      | 1212–1272| 61        | GCA       | –           | –          | −8                          |
| trnY    | N      | 1274–1334| 61        | GTA       | –           | –          | 1                           |
| cox1    | J      | 1333–2866| 1,534     | –         | CTG         | T          | −2                          |
| trnL2 (UUR) | J | 2867–2929 | 63 | TAA       | –           | –          | −5                          |
| cox2    | J      | 2930–3601| 672       | –         | ATA         | TAA        | 0                           |
| trnK    | J      | 3603–3674| 72        | CTT       | –           | –          | 1                           |
| trnD    | J      | 3675–3736| 62        | GTC       | –           | –          | 0                           |
| atp8    | J      | 3737–3844| 108       | –         | ATA         | TAA        | 0                           |
| atp6    | J      | 3841–4492| 652       | –         | ATA         | T          | −4                          |
| cox3    | J      | 4493–5273| 781       | –         | ATG         | T          | 0                           |
| trnG    | J      | 5274–5333| 60        | TCC       | –           | –          | 0                           |
| nad3    | J      | 5334–5684| 351       | –         | ATT         | TAG        | 0                           |
| trnA    | J      | 5683–5743| 61        | TGC       | –           | –          | −2                          |
| trnR    | J      | 5750–5809| 60        | TCG       | –           | –          | 6                           |
| trnN    | J      | 5808–5871| 64        | GTT       | –           | –          | −2                          |
| trnS1 (AGN) | J | 3871–3931 | 61 | GCT       | –           | –          | −1                          |
| trnE    | J      | 5931–5996| 66        | TGC       | –           | –          | −1                          |
| trnF    | N      | 5995–6056| 62        | GAA       | –           | –          | −2                          |
| nadS    | N      | 6059–7739| 1,681     | –         | GTG         | T          | −2                          |
| trnH    | N      | 7740–7803| 64        | GTG       | –           | –          | 0                           |
| nad4    | N      | 7804–9121| 1,318     | –         | ATG         | T          | 0                           |
| nad4l   | N      | 9115–9387| 273       | –         | ATG         | TAA        | −7                          |
| trnT    | J      | 9389–9451| 63        | TGT       | –           | –          | 1                           |
| trnP    | N      | 9451–9514| 64        | TGG       | –           | –          | −1                          |
| nad6    | J      | 9516–10008| 492 | –         | ATT         | T          | 1                           |
| cytb    | J      | 10009–11130| 1,122 | –         | ATG         | TAA        | 0                           |
| trnS2 (UCN) | J | 111130–11191| 62 | TGA       | –           | –          | −1                          |
| nadl    | N      | 11208–12123| 916 | –         | ATG         | T          | 16                          |
| trnL1 (CUN) | N | 12125–12186| 62 | TAG       | –           | –          | 1                           |
| rrnL    | N      | 12187–13392| 1,206 | –         | –           | –          | 0                           |
| trnV    | N      | 13393–13461| 69 | TAC       | –           | –          | 0                           |
| rrnS    | N      | 13462–14228| 767 | –         | –           | –          | 0                           |
| Control region | – | 14229–15259 | 1,031 | –         | –           | –          | 0                           |
Mitochondrial genomes usually show specific-strand bias in nucleotide composition, due to asymmetrical mutation pressure (Hassanin et al. 2005). In all compared species, the gene set on the J-strand was C-skewed and that on the N-strand was G-skewed. The comparison between AT bias on both strands indicated that the minority gene set was strongly T-skewed in each species, but the AT skew of majority gene set was different among the six compared species. In Ugyops sp., the gene set on the J-strand was moderately A-skewed (0.188). The AT skew was approximately zero in L. striatellus and C. velitchkovskyi, lacking significant A or T bias (Table 3), while the set of PCGs on the J-strand was subtly T-skewed in the remaining species.

For each codon of all PCGs, the second codon had lower AT content than the first and third codons in the six examined species. The first and second codons were T-skewed (Table 3). The value of AT skew at third codon position was positive in Ugyops sp. (0.053), whereas those were negative in other five delphacids.

### Table 3. Nucleotide composition of mitochondrial genomes in six species of Delphacidae

| Species | A + T content (%) | AT skew | GC skew |
|---------|------------------|---------|---------|
|         | Ugyops s. | S. f | P. m | N. l | L. s | C. v | Ugyops s. | S. f | P. m | N. l | L. s | C. v | Ugyops s. | S. f | P. m | N. l | L. s | C. v |
| Whole genome | 77.65 | 76.19 | 77.75 | 76.95 | 77.17 | 75.72 | 0.288 | 0.093 | 0.108 | 0.091 | 0.119 | 0.130 | -0.270 | -0.141 | -0.244 | -0.183 | -0.184 | -0.272 |
| All PCGs | 76.41 | 74.44 | 75.74 | 76.01 | 75.74 | 74.48 | -0.102 | -0.170 | -0.151 | -0.144 | -0.151 | -0.064 | -0.068 | -0.081 | -0.073 | -0.092 | -0.101 |
| J-strand PCGs | 74.83 | 72.27 | 73.98 | 74.14 | 73.52 | 72.55 | -0.189 | -0.044 | -0.012 | -0.031 | 0.0002 | 0.006 | -0.271 | -0.170 | -0.256 | -0.231 | -0.244 | -0.299 |
| N-strand PCGs | 78.93 | 77.89 | 78.54 | 78.99 | 79.30 | 77.57 | -0.541 | -0.355 | -0.359 | -0.344 | -0.359 | -0.385 | 0.328 | 0.136 | 0.255 | 0.237 | 0.218 | 0.284 |
| First codon | 73.15 | 71.52 | 73.47 | 73.56 | 72.82 | 73.02 | -0.008 | -0.032 | -0.052 | -0.014 | -0.022 | -0.031 | 0.166 | 0.139 | 0.131 | 0.099 | 0.118 | 0.112 |
| Second codon | 68.36 | 68.94 | 69.59 | 69.87 | 69.50 | 69.56 | -0.401 | -0.407 | -0.405 | -0.399 | -0.386 | -0.398 | -0.174 | -0.150 | -0.156 | -0.121 | -0.151 | -0.140 |
| Third codon | 87.74 | 82.86 | 84.17 | 84.59 | 84.92 | 80.84 | 0.053 | -0.092 | -0.027 | -0.079 | -0.052 | -0.047 | -0.283 | -0.262 | -0.292 | -0.273 | -0.354 | -0.339 |
| rRNAs | 78.50 | 76.46 | 78.45 | 77.83 | 77.83 | 76.74 | -0.274 | -0.082 | -0.094 | -0.076 | -0.076 | -0.105 | 0.302 | 0.267 | 0.308 | 0.298 | 0.318 | 0.335 |
| Control region | 88.86 | 82.50 | 86.15 | 79.29 | 83.20 | 80.12 | 0.055 | 0.004 | -0.025 | -0.007 | 0.028 | -0.006 | -0.096 | 0.105 | -0.231 | 0.169 | 0.294 | -0.130 |

Ugyops sp. (Ugyops), Sogatella furcifera (S. f), Peregrinus maidis (P. m), Nilaparvata lugens (N. l), Laodelphax striatellus (L. s), and Changeondelphax velitchkovskyi (C. v).

Mitochondrial genomes usually show specific-strand bias in nucleotide composition, due to asymmetrical mutation pressure (Hassanin et al. 2005). In all compared species, the gene set on the J-strand was C-skewed and that on the N-strand was G-skewed. The comparison between AT bias on both strands indicated that the minority gene set was strongly T-skewed in each species, but the AT skew of majority gene set was different among the six compared species. In Ugyops sp., the gene set on the J-strand was moderately A-skewed (0.188). The AT skew was approximately zero in L. striatellus and C. velitchkovskyi, lacking significant A or T bias (Table 3), while the set of PCGs on the J-strand was subtly T-skewed in the remaining species.

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### Protein-Coding Genes

The mitogenome of Ugyops sp. contained 13 PCGs typical to animal mitochondrial genomes. The canonical start codons ATN (Met/Ile) were assigned to 11 of all PCGs. Three genes (atp8, atp6, and cox2) started with ATA, three genes (nad2, nad3, and nad6) with ATT, and five genes (cox3, cytb, nad1, nad4, and nad4l) with ATG. The exceptions were cox1 and nad5, which used the noncanonical start codon CTG and GTG, respectively. In Hemiptera, employing GTG as start codon of nad5 was also found in the white-backed planthopper S. furcifera (Zhang et al. 2014) and the kissing bug...
Triatoma dimidiata (Dotson and Beard 2001). Furthermore, GTG was used as start codon of nad5 across a range of insect taxa, such as in some species of Diptera (Zhang et al. 2016b), Mecoptera (Beckenbach 2011), and Plecoptera (Stewart and Beckenbach 2006). Seven genes (atp6, cox1, cox3, nad1, nad4, nad5, and nad6) ended with incomplete stop codons T, which are presumably completed by polyadenylation after transcription (Ojala et al. 1981). The remaining genes had the complete termination codons TAA (atp8, cox2, cyt b, nad2, nad4l, and nad6), except for nad3, in which TAG was used.

The total number of codons was 3,612, excluding stop codons. Approximately equivalent codon numbers were detected in S. furcifera (3,606), C. velitchkovskyi (3,607), P. maidis (3,607), N. lugens (3,608), and L. striatellus (3,613). The three most abundant codon families were Phe, Met, and Ile (Fig. 2A), all of which were two-fold degenerate in codon usage and rich in A and T. When codons were calculated on the majority and minority strands separately, the most frequently used codon families were Met and Phe, respectively. The RSCU also reflected nucleotide compositional bias. Generally, codons with A or T in the third codon position were greatly preferred within each synonymous codon family, compared to codons with G or C in the third position. Both CCG (Pro) and UCG (Ser2 (UCN)) were lost in Ugyops sp. (Fig. 2B).

The average ratio of $K_a/K_s$ was calculated to evaluate the evolutionary rate of each PCG in the six delphacid mitogenomes. Among the 13 PCGs, nad4l had the highest rate (Fig. 3), followed by nad6.

Fig. 2. Codon distribution (A) and RSCU in the Ugyops sp. mitogenome (B). Codon Families are provided on the x-axis. CDspT, codons per thousand codons. Absent codons are marked at the top of bars.

Fig. 3. Evolutionary rates of 13 protein-coding genes in the mitogenomes of six delphacid species. The rate of nonsynonymous substitutions ($K_a$), the rate of synonymous substitutions ($K_s$), and the ratio of the rate of nonsynonymous substitutions to the rate of synonymous substitutions ($K_a/K_s$) are calculated for each PCG.
which located in the rearranged gene cluster trnT-trnP-nad6. Three lowest genes were cox1, cytb, and cox2, respectively ($K_a/K_s < 0.2$). For each PCG, the ratio of $K_a/K_s$ was less than 1, indicating the probable purifying selection in evolution of these genes. Furthermore, a negative correlation was detected between the $K_a/K_s$ ratio and the G + C content of each PCG ($R^2 = 0.867$, $P < 0.01$).

Fig. 4. Predicted secondary structures for the 22 tRNAs of the Ugyops sp. mitogenome. Watson–Crick pairs are indicated by lines, and wobble GU pairs are indicated by dots.
tRNAs and rRNAs

The length of all 22 tRNA genes ranged from 60 to 72 bp. The predicted secondary structures were typical cloverleaf except for trnS1 (AGN) (Fig. 4), in which the dihydrouridine (DHU) stem was replaced by a 6-bp simple loop. Similarly, trnS1 lacks the DHU arm in most other metazoans (Cameron 2014a). In Ugyops sp., the anticodon stem of trnV was longer than conservative length (5 bp), forming a 6-bp stem with an unpaired nucleotide. This type of oversize anticodon stem was also observed in trnS1 (AGN) of other hemipteran insects, including the aphid Cavariella salicicola (Wang et al. 2013) and some species of true bugs (Li et al. 2012, 2013, 2016; Yuan et al. 2015).

In total, 28 G–U wobble pairs were present in 10 acceptor stems, seven DHU stems, nine anticodon stems, and two TΨC stems of the tRNA secondary structures (Fig. 4). In addition, four mismatched pairs (5 A–A, 3 A–C, 2 A–G, and 10 U–U) were detected in the acceptor stem, the DHU stem, and the anticodon stem. Wobble and mismatched pairs, which are common in insect tRNAs, are usually corrected via editing processes (Lavrov et al. 2000).

The rrnL gene was 1,206 bp in size with an A + T content of 80.76%, while the rrnS gene is 767 bp long, with a little lower A + T content (74.93%). The secondary structure of rrnL of Ugyops sp. contained six structural domains (domain III is absent in arthropods) and 44 helices (Fig. 5). Helix H2735 at the 3′ end was not present, which was also absent in the leafhopper T. fasciana (Wang et al. 2017). Domains IV and V were more conserved than others according to sequence alignment of the six compared delphacid species. Four helices (H1775, H1830, H1935, and H2574) were most conserved with no more than one nucleotide substitution among the compared delphacid species. Some helices (H183, H235, H837, H991, and H2077) were highly variable in sequence and secondary structure.

The secondary structure of rrnS consisted of three domains and 27 helices (Fig. 6). Domain I and II were less conserved than domain III. Two helices H511 and H769 were most conserved among the compared species of Delphacidae. In domain III, different possible secondary structures could be predicted from the region including H1047, H1068, H1074, and H1113, because of several noncanonical base pairs (Gillespie et al. 2006, Cameron and Whiting 2008). The helix H1068 has been absent in some hemipteran species (e.g., Wang et al. 2013, 2017; Yuan et al. 2015), while this helix was identified in Ugyops sp.

Overlapping Sequences and Noncoding Regions

There were 12 overlaps (33 bp) found in the Ugyops sp. mitocnome (Table 2), and the longest one (8 bp) occurred between trnW and trnC, which oriented on different strands. In many insects, nad4l-nad4 and atp8-atp6 always overlap by 7 bp (ATGNTAA) in different reading frames (Stewart and Beckenbach 2005). The nad4l-nad4 overlap was almost identical in the six delphacid species, but the atp8-atp6 overlap was different in size (Fig. 7). In Ugyops sp., P. maidis and N. lugens, a 4-bp overlap (ATAA) was observed between atp8 and atp6, whereas the atp8-atp6 overlap (ATRTTAA) was 7 bp in other three species.
In total, 10 noncoding regions were spread throughout the mitogenome of *Ugyops* sp., including nine intergenic spacers (1–16 bp), and the control region (Table 2). The intergenic spacer between trnS2 *(UCN)* and *nad1* is common to many insects (Cameron and Whiting 2008), and it corresponds to the binding site of a transcription termination peptide (Roberti et al. 2003) and has a highly conserved 7-bp
motif that is conserved across insects (Cameron 2014b). In Ugyops sp., this spacer was 16 bp in length, while it was 17 bp long in other five species. The corresponding motif was TTACTTA in Ugyops sp., and TACTMR in other examined species of the subfamily Delphacinae (Fig. 8). The control region was the largest noncoding region in the mitogenome of Ugyops sp. and spanned 1,031 bp, located between rrnS and trnI. The A + T content (88.85%) of this region was higher compared with that of the whole mitogenome (77.65%). Three parts were recognized in the control region of Ugyops sp. as given in Fig. 9A: a 20-bp poly-thymidine (poly-T) stretch downstream of rrnS, a subregion of higher A + T content, and a tandem repeat sequence. The higher A + T content subregion (504 bp) was heavily biased toward A + T (94.05%) and included four microsatellite-like elements (TAAA)ₙ, (TA)ₙ, (TA)ₙ, and (TA)ₙ.

We compared the poly-T stretches and repeat sequences among the six delphacids. In the five species of Delphacinae, the poly-T stretch was 23 bp in length, longer than that found in Ugyops sp. (Fig. 9B). Despite length variations, the poly-T stretch seemed to be conserved in Delphacidae.

Tandem repetition has been frequently found in the control regions of insect mitogenomes (Zhang and Hewitt 1997). It has been proposed that the occurrence and persistence of tandem repeat units results from slipped-strand mispairing during mitochondrial DNA replication (Moritz et al. 1987, Macey et al. 1998). Tandem repeat sequences were detected in mitogenomes from all suborders of Hemiptera (Li and Liang 2018). In the six examined species of Delphacidae, repeat units occurred multiple times (Fig. 9C). A 21-bp consensus motif (AAAAATCGACAAAAAGAAAAAC) repeated 4.8 times in the control region of Ugyops sp., four complete units and a partial copy (16 bp) near trnI. The size of repeat unit varied in P. maidis, ranging from 20 to 22 bp (Fig. 9C). The repeat units of the remaining species were similar in both sequence and second structure (Fig. 9D). Particularly, the repeat unit of S. furcifera was identical to that of L. striatellus (Zhang et al. 2014). It was presumed that the subfamily Delphacinae has undergone a substantial radiation associated with host plant divergence (Urban et al. 2010, Huang et al. 2017), to which the similarity of repeat units might be related in the five species of Delphacinae. The sequence homology between Ugyops sp. and five Delphacinae species seemed limited (Fig. 9B), which might imply that evolution of control region in Delphacidae is very complicated. Further investigations of additional delphacid species from different groups would likely to provide useful information for understanding the way repeat units evolve in control region.

Fig. 7. Sequence alignments of atp8/atp6 and nad4l/nad4l in six species of Delphacidae.

Fig. 8. Alignments of the intergenic spacer between nad1 and trnS2 (UCR) in six species of Delphacidae.
Phylogenetic Analyses

The topology of ML tree was consistent with that of BI tree. In both analyses (Fig. 10), Delphacidae was monophyletic (bootstrap = 100, posterior probability = 1.00) and sister group to Cixiidae (represented by Pentastiridius sp.). In Delphacidae, two clades were strongly supported (bootstrap = 91, posterior probability = 1.00), the Ugyops sp. clade and the Delphacinae clade (Fig. 10). In the Delphacinae clade, C. velitchkovskyi, L. striatellus, S. furcifera, and N. lugens clustered together, indicating their relatively close relationships, which was likely supported by their similar tandem repeat unit in control regions.

Although the findings of the current study improved our understanding of the mitogenomics of the basal group Asiracinae, the
other subfamilies aside from Delphacinae remain poorly known. Additional taxonomic sampling will be needed to explore the diversity of their mitochondrial genomes and provide more complete insights into the evolution of Delphacidae.

**Supplementary Data**

Supplementary data are available at *Journal of Insect Science* online.

**Acknowledgments**

We wish to thank Zhi Shun Song for collecting specimens and Qi Qi Wang, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, for giving advice on prediction of rRNA secondary structures. We are grateful to Michael C. Orr, Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, for offering suggestions on writing. The work on which this paper is based was supported by the Cooperative Research Projects between the Chinese mainland and Taiwan in Biodiversity jointly supported by the National Natural Science Foundation of China and the K.T. Li Foundation for the Development of Science and Technology (grant 31561163003) and the National Natural Science Foundation of China (grant 31572298).

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