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Research

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

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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 codon, 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 rRNA gene (*rrnS*), a subregion of higher A + T content and tandemly repeated sequence near *trnI*. 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 phylogenomics 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: *Changeondelphax velitchkovskyi*, *Laodelphax striatellus*, *Nilaparvata lugens*, *Peregrinus maidis*, and *Sogatella furcifera*. Moreover, gene rearrangements are known for these species, with two clusters *trnW-trnC-trnY* and *trnT-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 phytoplasma, 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 MITOS Web Server (Bernt et al. 2013). PCGs were identified using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) under the invertebrate mitochondrial genetic code. For some PCGs, start and stop codons were corrected according to alignment of homologous genes in mitogenomes of Auchenorrhyncha. The beginning and end of the *rrnL* gene were presumed to extend to the boundaries of the adjacent tRNA genes *trnL1* (*CUN*) and *trnV*. The 5′ end of *rrnS* gene was determined by aligning *rrnS* sequences of auchenorrhynchan mitogenomes, and the 3′ end was assumed to be delimited by the beginning of *trnV*. 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), *Panaorus 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 (K_s), the number of nonsynonymous substitutions per nonsynonymous site (K_a), and the ratio of K_a/K_s for each PCG. The repeat motifs in the control region were detected using Tandem Repeats Finder (Benson 1999). Comparation 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 PartitonFinder 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://iqtree.

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

	Superfamily	Family	Species	Accession number
Ingroup	Fulgoroidea	Delphacidae	*Changeondelphax velitchkovskyi*	MG049916
			Laodelphax striatellus	JX880068
			Nilaparvata bakeri	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
		Ricaniidae	*Ricania speculum*	NC_031369
	Cercopoidea	Aphrophoridae	*Philaenus spumarius*	NC_005944
		Cercopidae	*Abidama producta*	NC_015799
			Callitettix braconoides	NC_025497

cibiv.univie.ac.at/) 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, Plesiodelphacinae, 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	65–131	67	TTG	–	–	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	5871–5931	61	GCT	–	–	−1
trnE	J	5931–5996	66	TTC	–	–	−1
trnF	N	5995–6056	62	GAA	–	–	−2
nad5	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	11130–11191	62	TGA	–	–	−1
nad1	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

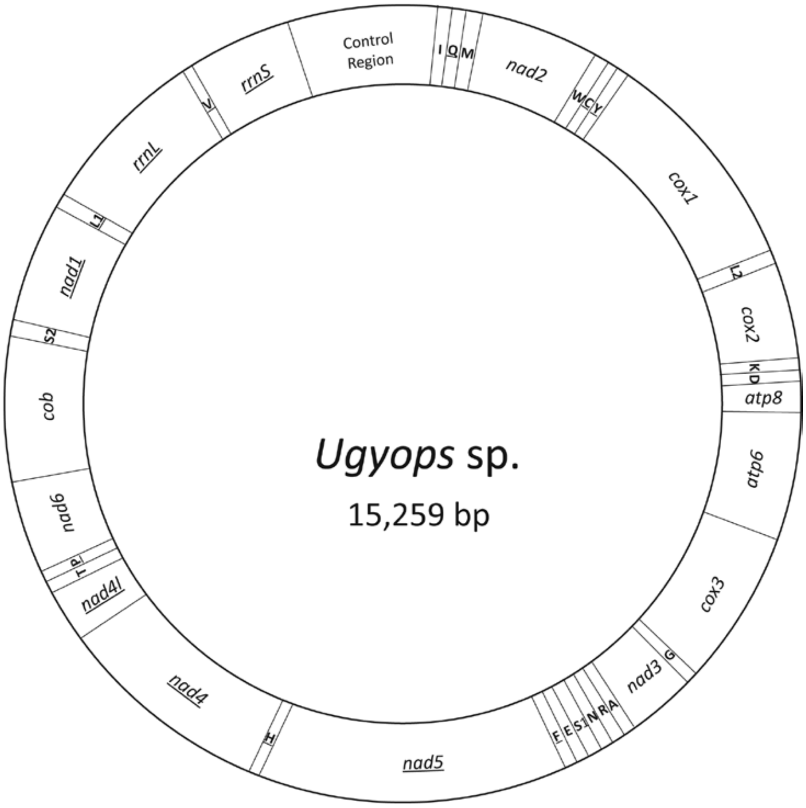

Fig. 1. Structure of the mitochondrial genome of *Ugyops* sp.

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

Species	A + T content (%)						AT skew						GC skew					
	Ugyops	*S. f*	*P. m*	*N. l*	*L. s*	*C. v*	*Ugyops*	*S. f*	*P. m*	*N. l*	*L. s*	*C. v*	*Ugyops*	*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	−0.156	−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.

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.

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*, *cytb*, *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*

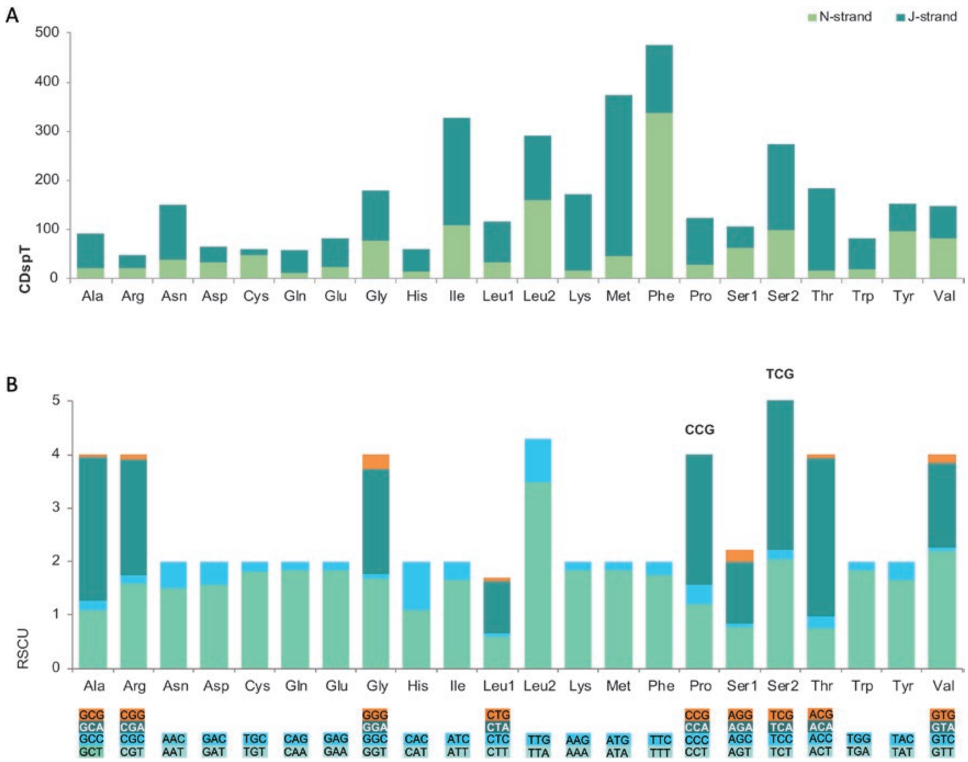

Fig. 2. Codon distribution (A) and RSCU in the *Ugyops* sp. mitogenome (B). Codon Families are provided on the x-axis. CDspT, codons per thousand codons. Absent codons are marked at the top of bars.

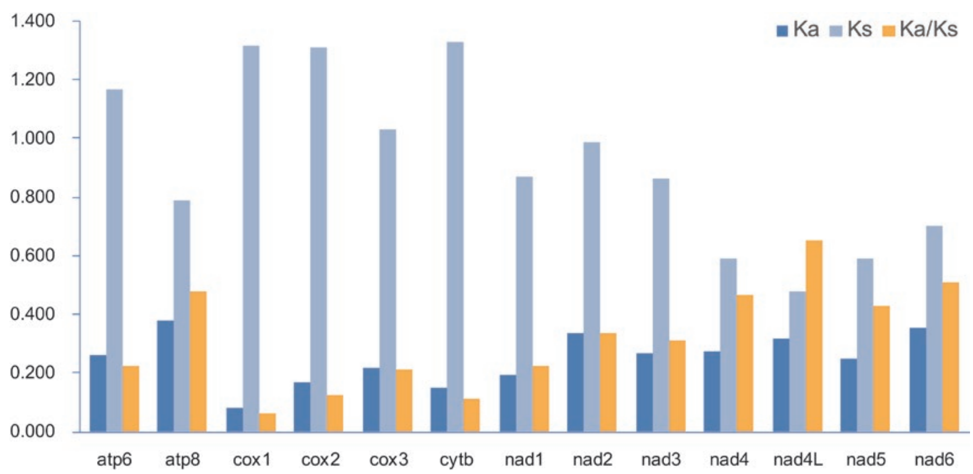

Fig. 3. Evolutionary rates of 13 protein-coding genes in the mitogenomes of six delphacid species. The rate of nonsynonymous substitutions (K_a), the rate of synonymous substitutions (K_s), and the ratio of the rate of nonsynonymous substitutions to the rate of synonymous substitutions (K_a/K_s) are calculated for each PCG.

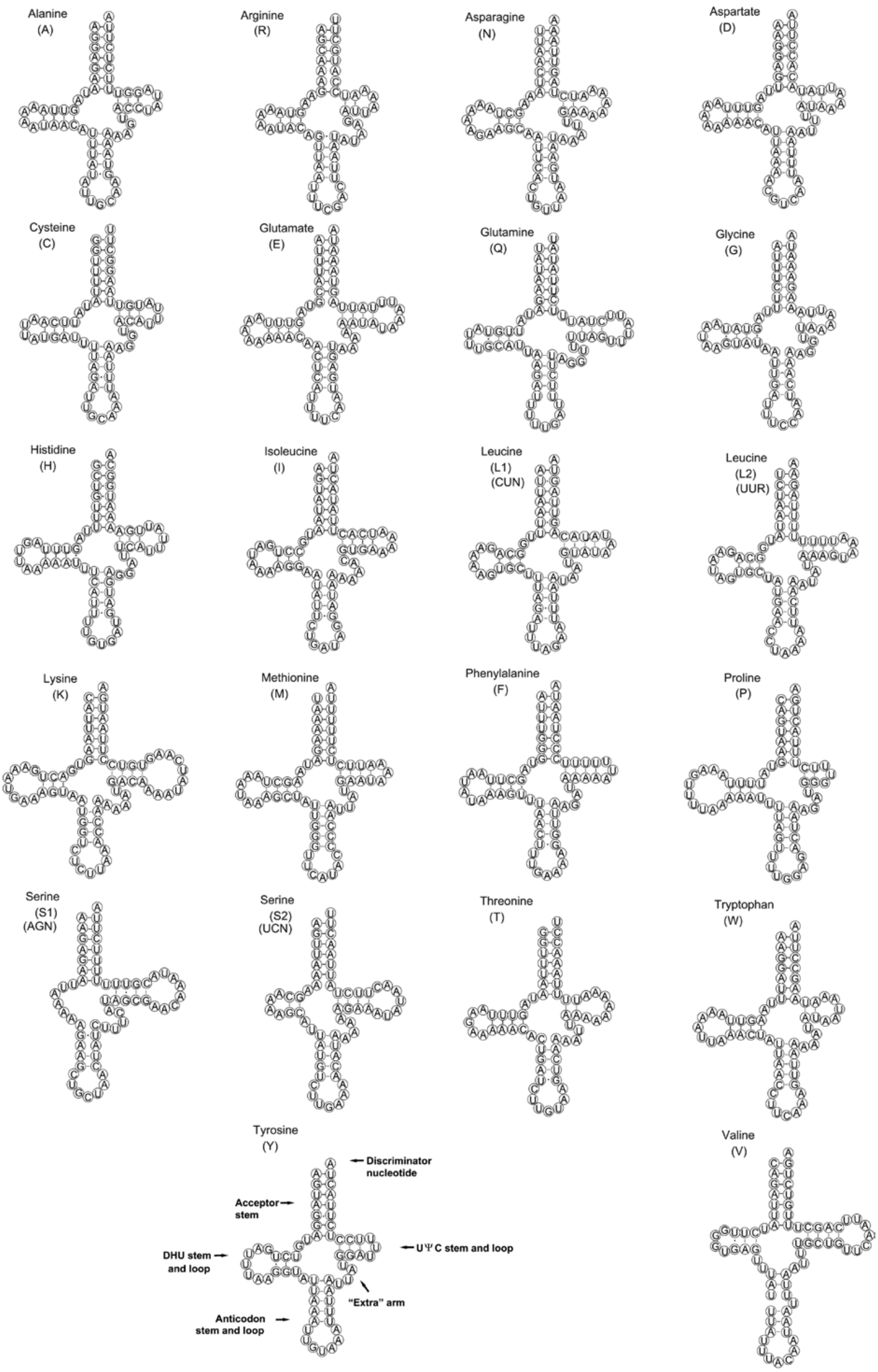

Fig. 4. Predicted secondary structures for the 22 tRNAs of the *Ugyops* sp. mitogenome. Watson–Crick pairs are indicated by lines, and wobble GU pairs are indicated by dots.

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 ($R^2 = 0.867$, $P < 0.01$).

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 oversized 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 delphacids. 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. mitogenome (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.

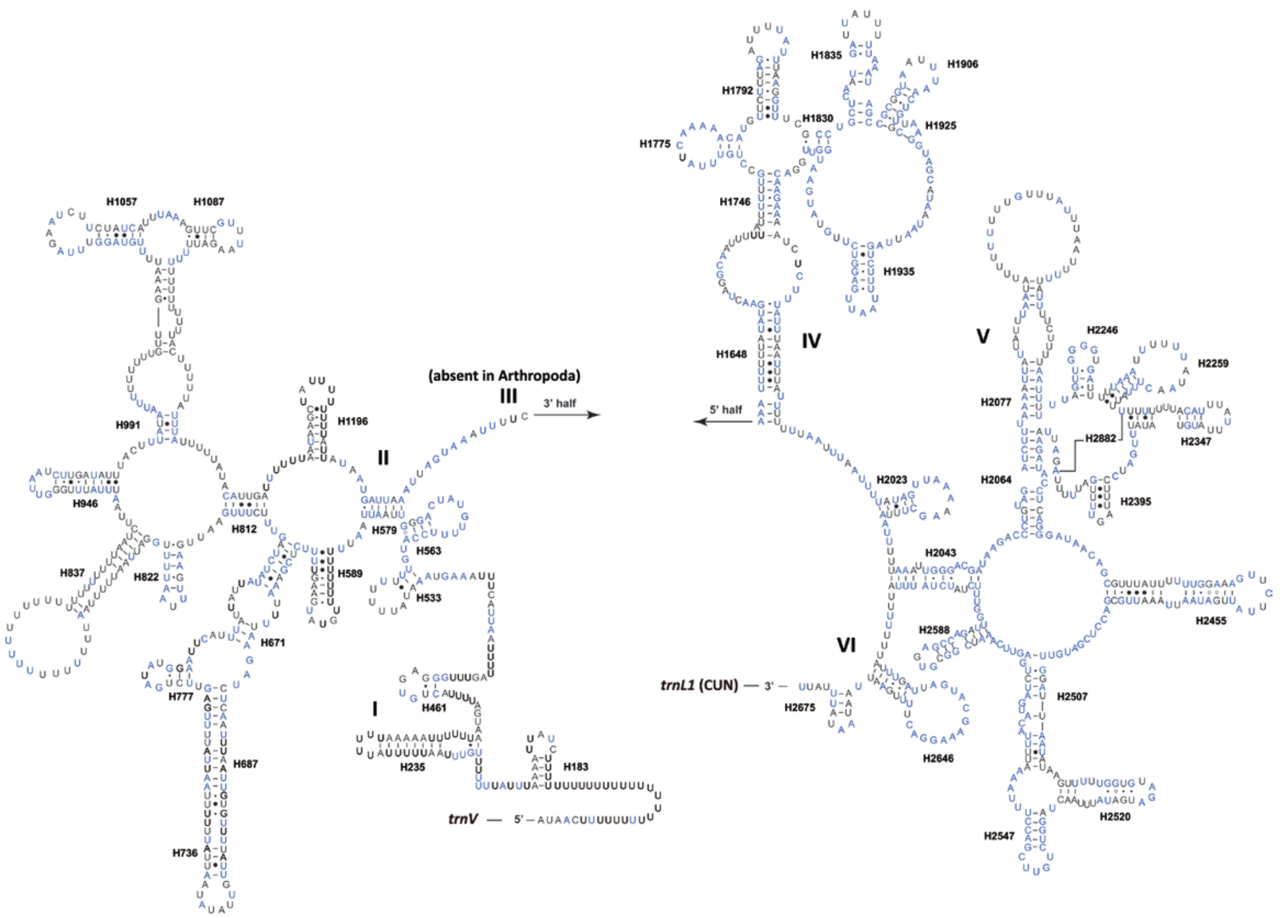

Fig. 5. Predicted secondary structure for the *rrnL* in the mitogenome of *Ugyops* sp. Base pairing is illustrated as follows: Watson–Crick pairs by lines; wobble GU pairs by dots; AG pairs by circles; other noncanonical pairs by solid circles. The 100% identical nucleotides in the six compared species of Delphacidae are marked in blue.

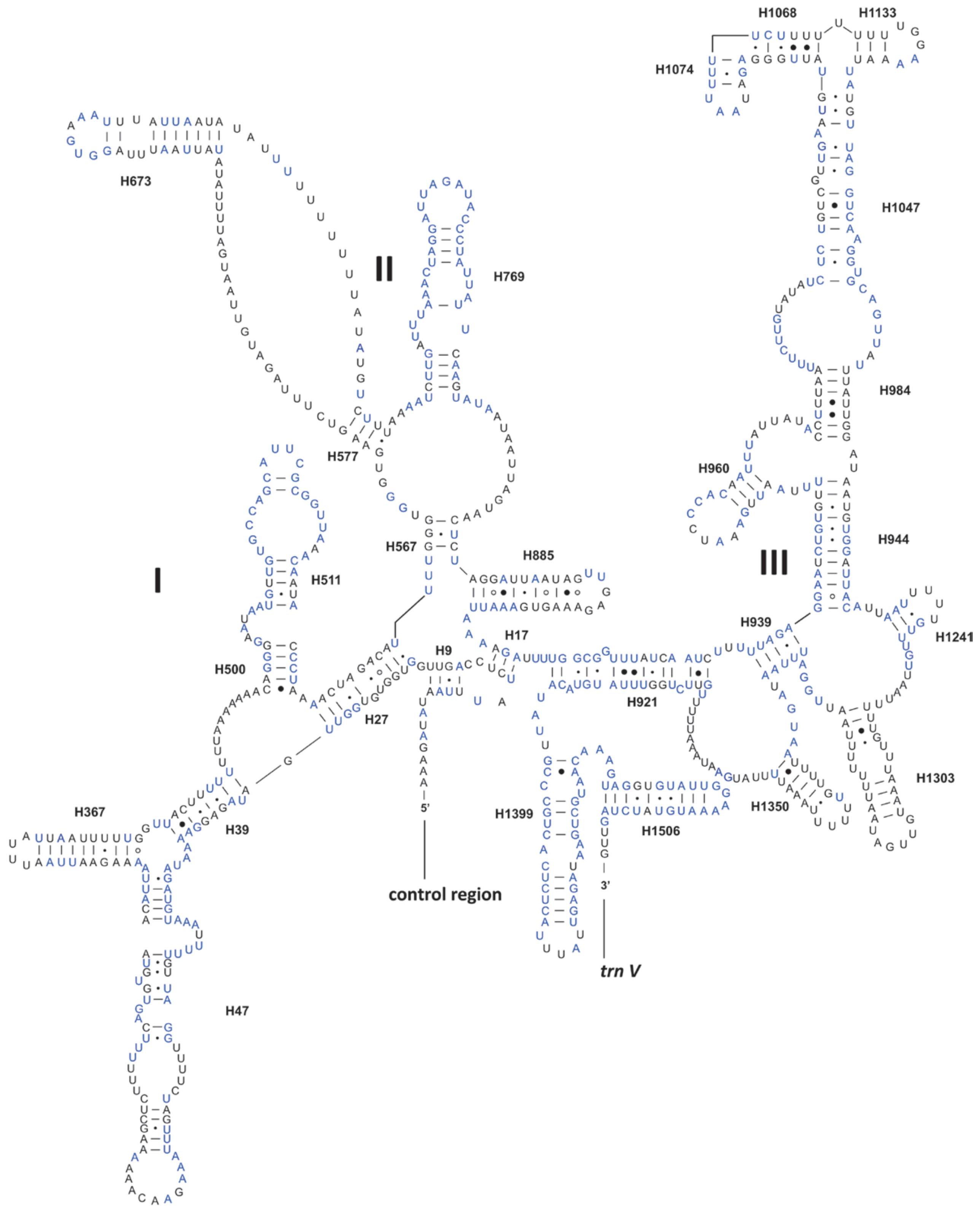

Fig. 6. Predicted secondary structure for the *rrnS* in the mitogenome of *Ugyops* sp. Base pairing is illustrated as follows: Watson–Crick pairs by lines; wobble GU pairs by dots; AG pairs by circles; other noncanonical pairs by solid circles. The 100% identical nucleotides in the six compared species of Delphacidae are marked in blue.

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 $(TAAA)_3$, $(TA)_8$, $(TA)_9$, and $(TA)_{10}$.

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 (AAAAATCGACCAAAAGAACAC) 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 *nad4*/*nad4l* in six species of Delphacidae.

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

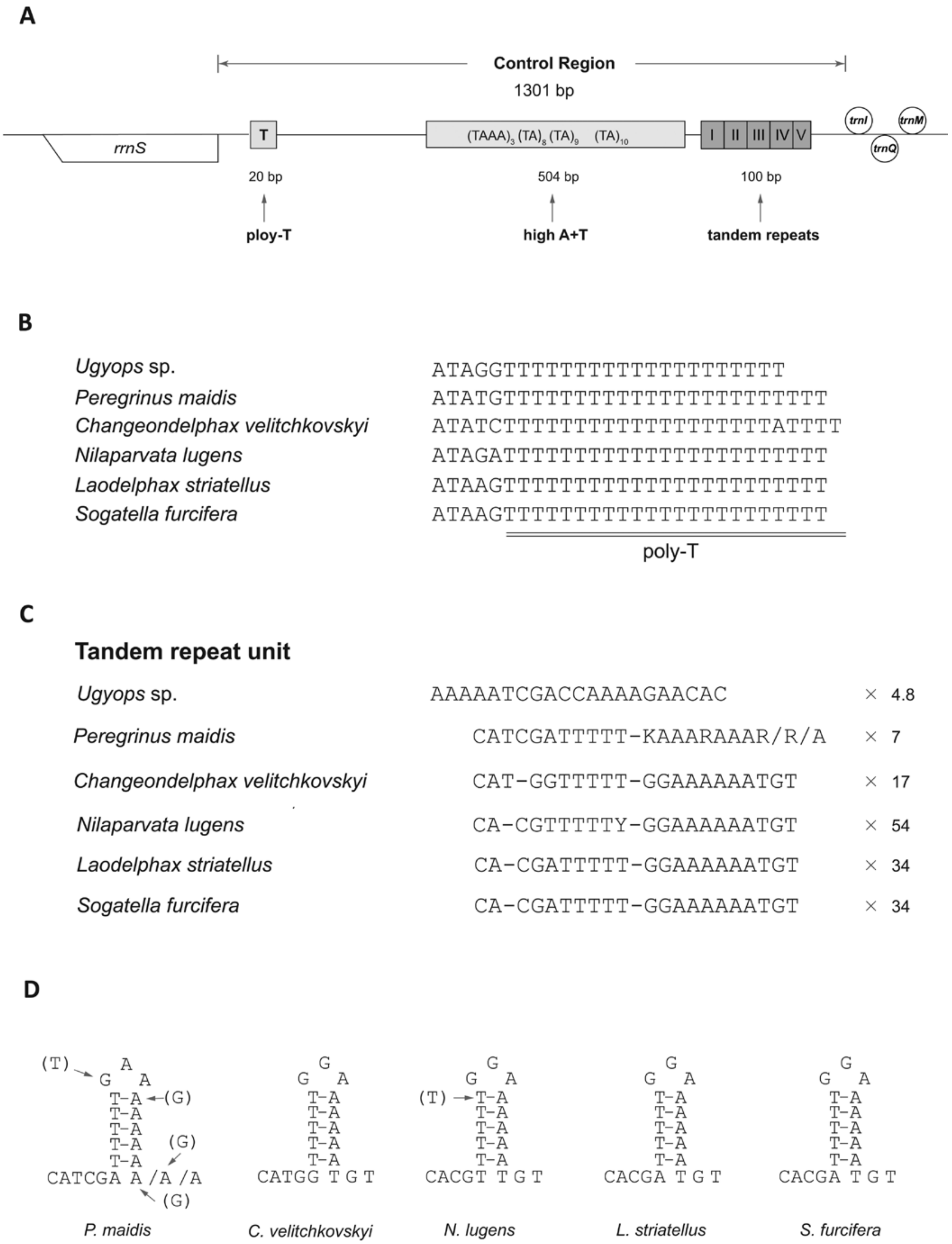

Fig. 9. Control region of *Ugyops* sp. mitogenome, and the comparison of two elements in control regions of six delphacid species. (A) Map of the control region in *Ugyops* sp. (B)The poly-T stretch in six species of Delphacidae. (C) Sequences of tandem repeat unit in the six examined species of Delphacidae. (D) Predicted secondary structures of tandem repeat unit.

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

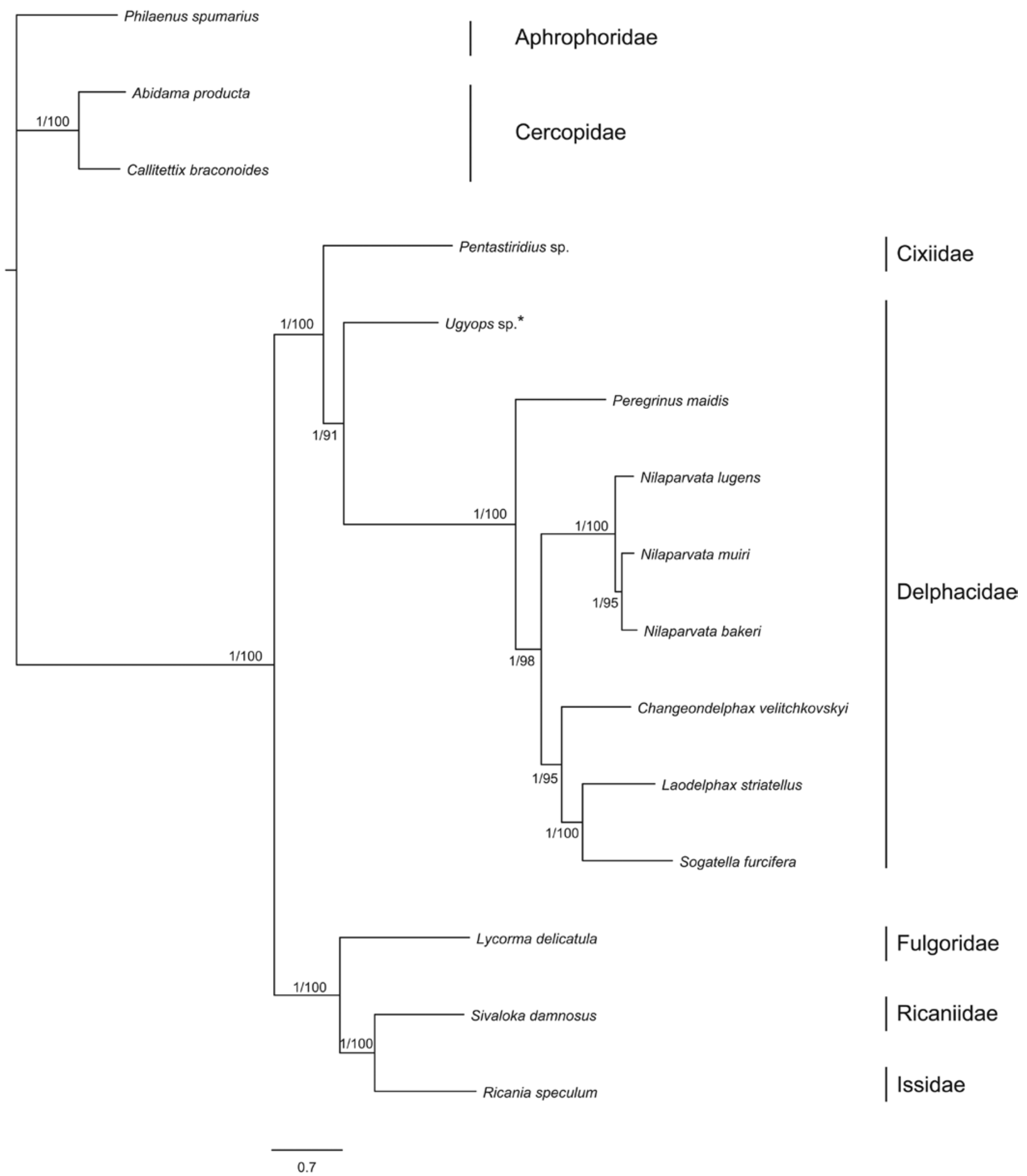

Fig. 10. Phylogenetic tree inferred from ML and BI using the data set of 13 PCGs. Nodal supports are indicated above or below the branches.

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.

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