International Journal of Biological Macromolecules 119 (2018) 60–69

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Characterization of the complete mitochondrial genome of *Phymatostetha huangshanensis* (Hemiptera: Cercopidae) and phylogenetic analysis

Tianjuan Su, Aiping Liang *

Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 19 June 2018
Received in revised form 17 July 2018
Accepted 20 July 2018
Available online 21 July 2018

Keywords:
Cercopoidea
Mitochondrial genome
Phylogeny

ABSTRACT

The circular mitochondrial genome (mitogenome) of *Phymatostetha huangshanensis* is 17,785 bp long. It contains the typical set of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and a large control region. The gene organization, nucleotide composition, and codon usage are similar to other Cercopoidea mitogenomes. However, the control region, including multiple types of tandem repeats, is longer than those of other spittlebugs. All PCGs initiate with standard start codon of ATN or TTG and share the complete stop codon of TAA or TAG, whereas *cox2* and *cox3* end with a single T. All tRNAs have the typical clover-leaf structure except for *trnS1*. In addition, the unpaired nucleotide is detected in the anticodon stem of *trnS1* and the acceptor stem of *trnR*. The secondary structures of *rrnL* and *rrnS* comprise 44 helices and 27 helices, respectively. Phylogenetic analysis is performed on the 13 PCGs and two rRNAs of 24 Cicadomorpha mitogenomes. Both the maximum likelihood and Bayesian methods robustly support the relationships of (Membracoidea + (Cicadoidea + Cercopoidea)). Within Cercopoidea, the monophyly of Cercopidae is also supported. Furthermore, we firstly present the taxonomic status of *Phymatostetha* with the relationships of (*Cosmoscarta* + (*Phymatostetha* + *Paphnutius*)).

1. Introduction

The superfamily Cercopoidea (Hemiptera: Cicadomorpha) encompasses over 3000 species distributed among approximately 340 genera in five families (Aphrophoridae, Cercopidae, Clastopteridae, Epipygidae, and Machaerotidae) [1]. They are also called spittlebugs as their nymphal habit of producing copious spittle masses to cover themselves inside through continuously sucking liquid and nutrient contained in xylem tissue. Accordingly, many Cercopoidea species cause heavy economic damage to host plants. Within the family Cercopidae, some species were also demonstrated to aggregate in one spittle mass and inflict more serious economic damage [2]. The genus *Phymatostetha* has been reported as the pests of banana [3, 4]. However, some taxa are difficult to identify to species due to insufficient diagnostic features. Even less is known about the taxonomic status of *Phymatostetha* within Cercopoidea and its phylogenetic relationship with other spittlebugs. To date, only very limited molecular data focused on *Phymatostetha* are available. There have been no studies on the mitochondrial genome (mitogenome), which could systematically provide valuable information for species identification, taxonomic status, and phylogenetic analysis.

The typical insect mitogenome is circular and compact, ranging from 14 to 20 kb. It has relatively stable gene organization, containing 13 protein coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and a A + T-rich region (also known as the control region) [5–7]. Although both the gene order and direction of insect mitogenomes are variable among different taxa [8], the previously sequenced Cercopoidea mitogenomes share the identical gene arrangement with the ancestral type of insect [9, 10]. Owing to some unique features like its small size, maternal inheritance, low rate of recombination, and accelerated rate of nucleotide substitution [11, 12], mitogenome has been extensively used in various study areas, including species delimitation, molecular evolution, phylogenetic inference, and population genetics [13–15]. Furthermore, structural genomic features, such as the secondary structures of tRNA and rRNAs, have also been extensively used for comparative and evolutionary genomics [6, 9, 15, 16].

To date, there are only ten complete mitogenomes have been sequenced for Cercopoidea, which is quite limited and restricts our understanding of evolution in Cercopoidea species at the genomic level. To provide additional Cercopoidea resources and clarify their phylogenetic relationships, the complete mitogenome of *P. huangshanensis* was sequenced. The genomic structure and composition was analyzed,

* Corresponding author at: Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China.
E-mail address: liangap@ioz.ac.cn (A. Liang).

https://doi.org/10.1016/j.ijbiomac.2018.07.135
0141-8130/

Table 1
Summary of mitogenomes used in this study.

Superfamily	Family	Species	Size (bp)	GenBank no.
Cercopoidea	Cercopidae	*Cosmoscarta bispecularis*	15,426	KP064511
	Cercopidae	*Cosmoscarta* sp.	15,651	KF621236
	Cercopidae	*Callitettix braconoides*	15,637	NC_025497
	Cercopidae	*Callitettix biformis*	15,222	NC_025496
	Cercopidae	*Callitettix versicolor*	15,374	EU725832
	Cercopidae	*Aeneolamia contigua*	15,613	NC_025495
	Cercopidae	*Abidama producta*	15,277	NC_015799
	Cercopidae	*Phymatostetha huangshanensis*	17,045	MG878381
	Cercopidae	*Paphnutius ruficeps*	14,841	NC_021100
	Aphrophoridae	*Philaenus spumarius*	16,324	NC_005944
Cicadoidea	Cicadidae	*Tettigades auropilosa*	14,944	KM000129
	Cicadidae	*Magicicada tredecim*	14,844	KM000130
	Cicadidae	*Diceroprocta semicincta*	14,920	KM000131
Membracoidea	Membracidae	*Entylia carinata*	15,662	NC_033539
	Membracidae	*Leptobelus gazella*	16,007	NC_023219
	Aetalionidae	*Darthula hardwickii*	15,355	NC_026699
	Cicadellidae	*Macrosteles quadrilineatus*	16,626	NC_034781
	Cicadellidae	*Tambocerus* sp.	15,955	KT827824
	Cicadellidae	*Nephotettix cincticeps*	14,805	NC_026977
	Cicadellidae	*Bothrogonia ferruginea*	15,262	KU167550
	Cicadellidae	*Homalodisca coagulata*	15,304	AY875213
	Cicadellidae	*Drabescoides nuchalis*	15,309	NC_028154
	Cicadellidae	*Idioscopus nitidulus*	15,287	NC_029203
	Cicadellidae	*Empoasca vitis*	15,154	NC_024838
Fulgoroidea	Delphacidae	*Nilaparvata muiri*	14,371	NC_024627
	Ricaniidae	*Ricania marginalis*	15,698	NC_019597

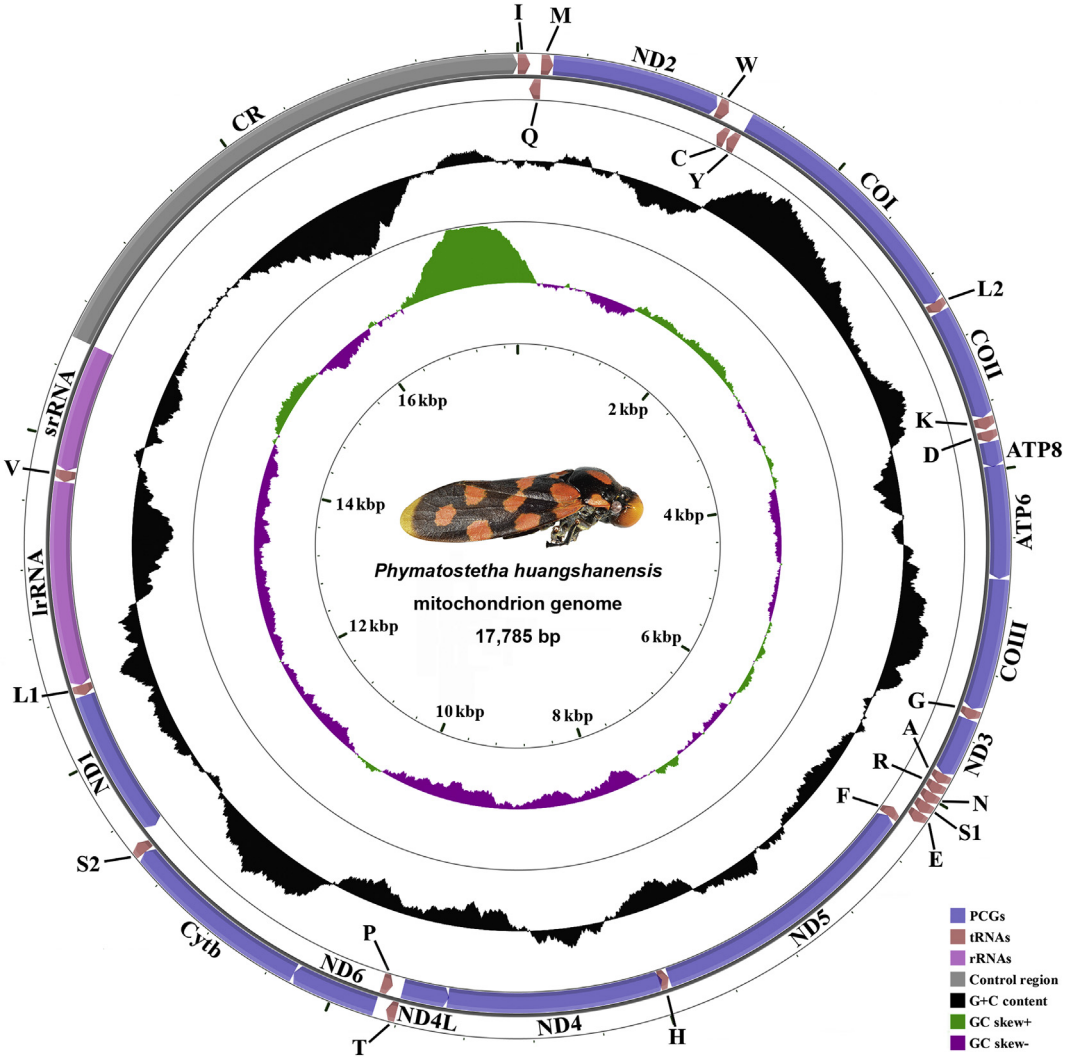

Fig. 1. Organization of the complete mitogenome of *P. huangshanensis*.

Table 2
Annotation of the *P. huangshanensis* mitogenome.

Gene	Direction	Position	Size	Start codon	Stop codon	Anticodon	Intergenic nucleotides
trnI	J	1–73	73			GAT	
trnQ	N	71–139	69			TTG	−3
trnM	J	139–209	71			CAT	−1
nad2	J	210–1205	996	TTG	TAA		0
trnW	J	1211–1281	71			TCA	5
trnC	N	1274–1336	63			GCA	−8
trnY	N	1342–1409	68			GTA	5
cox1	J	1413–2951	1539	ATG	TAA		3
trnL2(UUR)	J	2947–3012	66			TAA	−5
cox2	J	3013–3685	673	ATA	T		0
trnK	J	3686–3756	71			CTT	0
trnD	J	3767–3831	65			GTC	10
atp8	J	3832–3981	150	ATT	TAA		0
atp6	J	3975–4640	666	ATG	TAA		−7
cox3	J	4644–5424	781	ATG	T		3
trnG	J	5425–5487	63			TCC	0
nad3	J	5488–5841	354	ATA	TAA		0
trnA	J	5841–5903	63			TGC	−1
trnR	J	5904–5970	67			TCG	0
trnN	J	5971–6036	66			GTT	0
trnS1(AGN)	J	6036–6104	69			GCT	−1
trnE	J	6105–6168	64			TTC	0
trnF	N	6167–6230	64			GAA	−2
nad5	N	6230–7942	1713	TTG	TAA		−1
trnH	N	7943–8005	63			GTG	0
nad4	N	7986–9326	1341	ATG	TAA		−20
nad4L	N	9320–9610	291	ATG	TAA		−7
trnT	J	9613–9675	63			TGT	2
trnP	N	9676–9742	67			TGG	0
nad6	J	9744–10,259	516	ATT	TAA		1
cytb	J	10,259–11,392	1134	ATG	TAG		−1
trnS2(UCN)	J	11,391–11,460	70			TGA	−2
nad1	N	11,479–12,396	918	ATG	TAA		18
trnL1(CUN)	N	12,398–12,465	68			TAG	1
rrnL	N	12,466–13,734	1269				0
trnV	N	13,735–13,806	72			TAC	0
rrnS	N	13,807–14,586	780				0
Control region		14,587–17,785	3199				0

including the nucleotide composition, codon usage, secondary structures of tRNA and rRNA genes, and the tandem repeats of control region. Furthermore, the mitogenomic phylogeny of Cicadomorpha was reconstructed, with the conformation of the taxonomic status of *Phymatostetha*.

2. Materials and methods

2.1. Sample and DNA extraction

The specimen of *P. huangshanensis* was collected in Qiyun Mountain, Jiangxi Province, China (N25°49′56″, E114°01′55″). It was preserved in absolute ethyl alcohol and stored in −20 °C freezer until use. Total genomic DNA was extracted from legs of the specimen using a DNeasy Blood & Tissue kit (Qiagen Hilden, Germany) following the manufacturer's instructions. Both the specimen and its DNA were stored at the Group of Cicadas, Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences.

Table 3
Composition and skewness in the *P. huangshanensis* mitogenome.

Regions	Size	A%	G%	T%	C%	A + T %	G + C %	AT skew	GC skew
Whole genome	17,785	44.3	9.7	34.4	12.5	77.8	22.2	0.115	−0.129
PCGs	11,072	43.3	9.6	33.0	14.1	76.3	23.7	0.135	−0.189
tRNA genes	1476	43.4	9.4	35.8	11.3	79.3	20.7	0.096	−0.092
rRNA genes	2049	49.8	7.1	30.3	12.8	80.1	19.9	0.243	−0.289
Control region	3199	39.7	11.7	41.3	7.4	80.9	19.1	−0.020	0.226

2.2. PCR amplification and sequencing

The complete mitogenome was amplified with eight pairs of primers, which were designed according to the conserved sequence regions of previously reported Cercopoidea mitogenomes (Table S1). PCR amplification was performed using TaKaRa LA Taq (Takara Co., Dalian, China), with the following cycling steps: an initial denaturation for 2 min at 92 °C; followed by 35–40 cycles of 30 s at 92 °C, 30 s at 48–52 °C, and 12 min at 60 °C; and a final extension of 20 min at 60 °C. The PCR products were assessed using 1% agarose gel electrophoresis, purified, and sequenced with the PCR primers and internal primers generated by primer walking. Some fragments were also cloned into the pMDTM19-T vector (Takara Bio Inc., Dalian, China) and sequenced with the primers of M13-F and M13-R. All fragments were sequenced by Majorbio Biotechnology Company (Beijing, China) with a DNA sequencer of ABI 3730XL (PE Applied Biosystems, San Francisco, CA, USA).

2.3. Sequence analysis

Sequences were assembled using BioEdit 7.0.9.0 [17] and SeqMan program included in the Lasergene software package (DNAStar Inc., Madison, Wisc.). The transfer RNA genes (tRNAs) were identified using the Mitos WebServer [18]. The positions of protein-coding genes (PCGs), ribosomal RNA genes (rRNAs) and control region were confirmed by the boundaries of tRNAs and by sequence comparison with other Cercopoidea mitogenomes. PCGs were also translated into

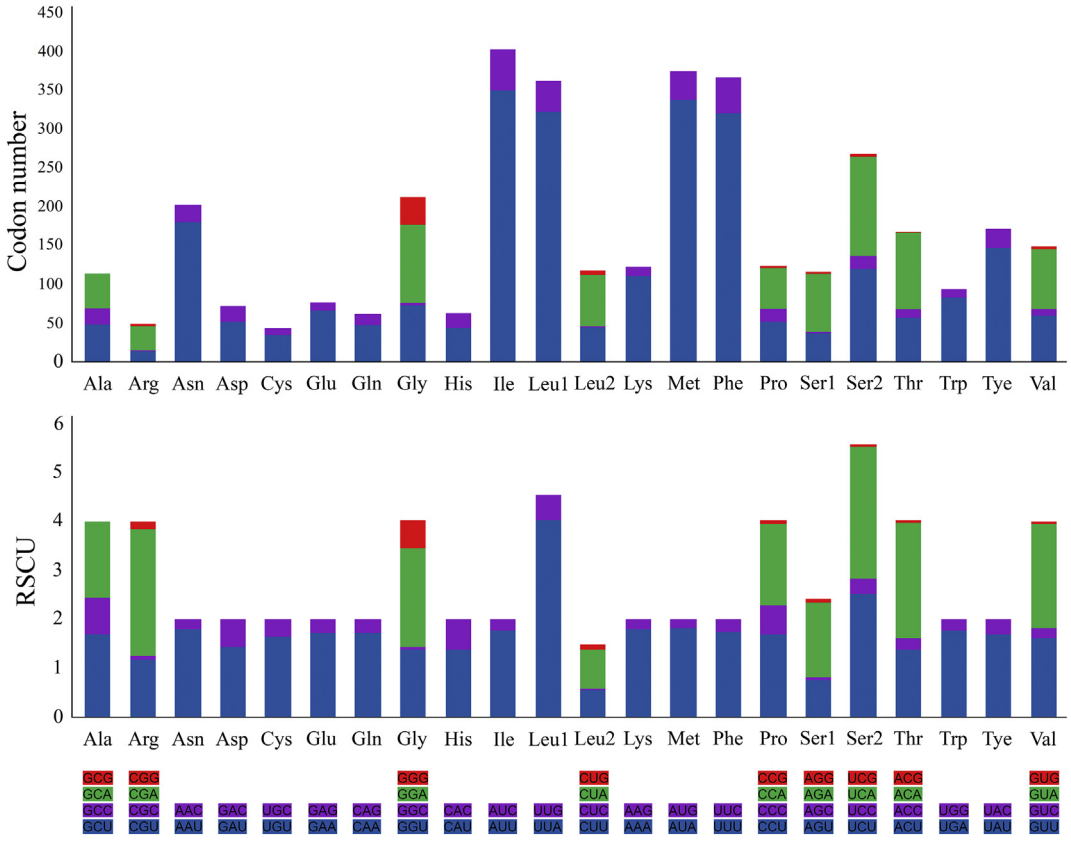

Fig. 2. The codon number and relative synonymous codon usage (RSCU) of PCGs in *P. huangshanensis* mitogenome.

amino acids based on the invertebrate mitochondrial genetic code. The mitogenomic map was depicted with CGView Comparison Tool [19]. The base composition and relative synonymous codon usage (RSCU) were analyzed using MEGA 6.05 [20]. Composition skew analysis was calculated with the formulas: AT skew = (A − T) / (A + T) and GC skew = (G − C) / (G + C) [21]. The tandem repeats of the control region were identified by the tandem repeats finder online server [22]. The secondary structures of rRNA genes were inferred according to the patterns proposed for other hemipterans [23–26]. In addition, gene regions that lacking significant homology were also folded by the Mfold Web Server [27]. Helix numbering was named following the convention of the Comparative RNA Web (CRW) Site [28].

2.4. Phylogenetic analysis

Phylogenetic analysis was performed on the dataset of 13 PCGs and two rRNAs from 24 complete or nearly complete mitogenomes of Cicadomorpha, with two Fulgoromorpha species selected as outgroups (Table 1). MEGA 6.05 was used to align the nucleotide sequences of each PCG based on their amino acid sequences. The two rRNA genes were aligned with MAFFT 7.310 using the Q-INS-i algorithm [29]. To eliminate poorly aligned positions, Gblocks 0.91b [30] was used with the default settings except for the gap positions toggled as "none". These 15 alignments were concatenated by BioEdit 7.0.9.0., with the total length of 10,564 bp. The nucleotide substitution models and best-fit partitioning schemes (Table S2) were simultaneously recommended by PartitionFinder 2.1.1 [31] using the Bayesian Information Criterion (BIC).

The maximum likelihood (ML) analysis was conducted using IQ-TREE [32] as implemented on the IQ-TREE Web Server (http://iqtree.cibiv.univie.ac.at/). Branch support was estimated with 1000 replicates of ultrafast likelihood bootstrap. Bayesian inference (BI) analysis was performed on MrBayes 3.2.6 [33] through the online CIPRES Science gateway [34]. Two simultaneous runs were performed, each with one cold chain and three hot chains, with posterior distributions estimated using Markov Chain Monte Carlo (MCMC) sampling. The MCMC chains were set for 10,000,000 generations and sampled every 1000 steps, with a relative burn-in of 25%. The convergence of the independent runs was assessed by the average standard deviation of the split frequencies (<0.01). The phylogenetic tree was drawn with the software of FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

3. Results and discussion

3.1. Genome organization and nucleotide composition

The mitogenome of *P. huangshanensis* was completely sequenced, with the length of 17,785 bp (GenBank accession number MG878381). It was larger than any other reported Cercopoidea mitogenomes, which ranged from 14,841 bp in *Paphnutius ruficeps*, Cercopidae [35] to 16,324 bp in *Philaenus spumarius*, Aphrophoridae [36]. This mitogenome contained the entire set of 37 genes (13 PCGs, two rRNAs, and 22 tRNAs) and a control region. The majority strand (J-strand) carried most of the genes (nine PCGs and 14 tRNAs), while other genes (four PCGs, two rRNAs, and eight tRNAs) were encoded on the minority strand (N-strand) (Fig. 1; Table 2). All genes have the identical order and orientation with those of other Cercopoidea [37, 38]. Except for the large control region, the mitogenome of *P. huangshanensis* was relatively compact, which contained a total of 48 intergenic nucleotides distributed in 9 locations, ranging in size from 1 to 18 bp. In addition, this genome had 13 overlapping regions (1–20 bp) and 15 pairs of neighboring genes.

The overall base composition was A (43.3%), T (34.4%), C (12.5%), and G (9.7%) on the J-strand, showing the biased usage of A + T nucleotides (77.8%) (Table 3). Composition skew analysis revealed that *P. huangshanensis* had positive AT skew and negative GC skew, not

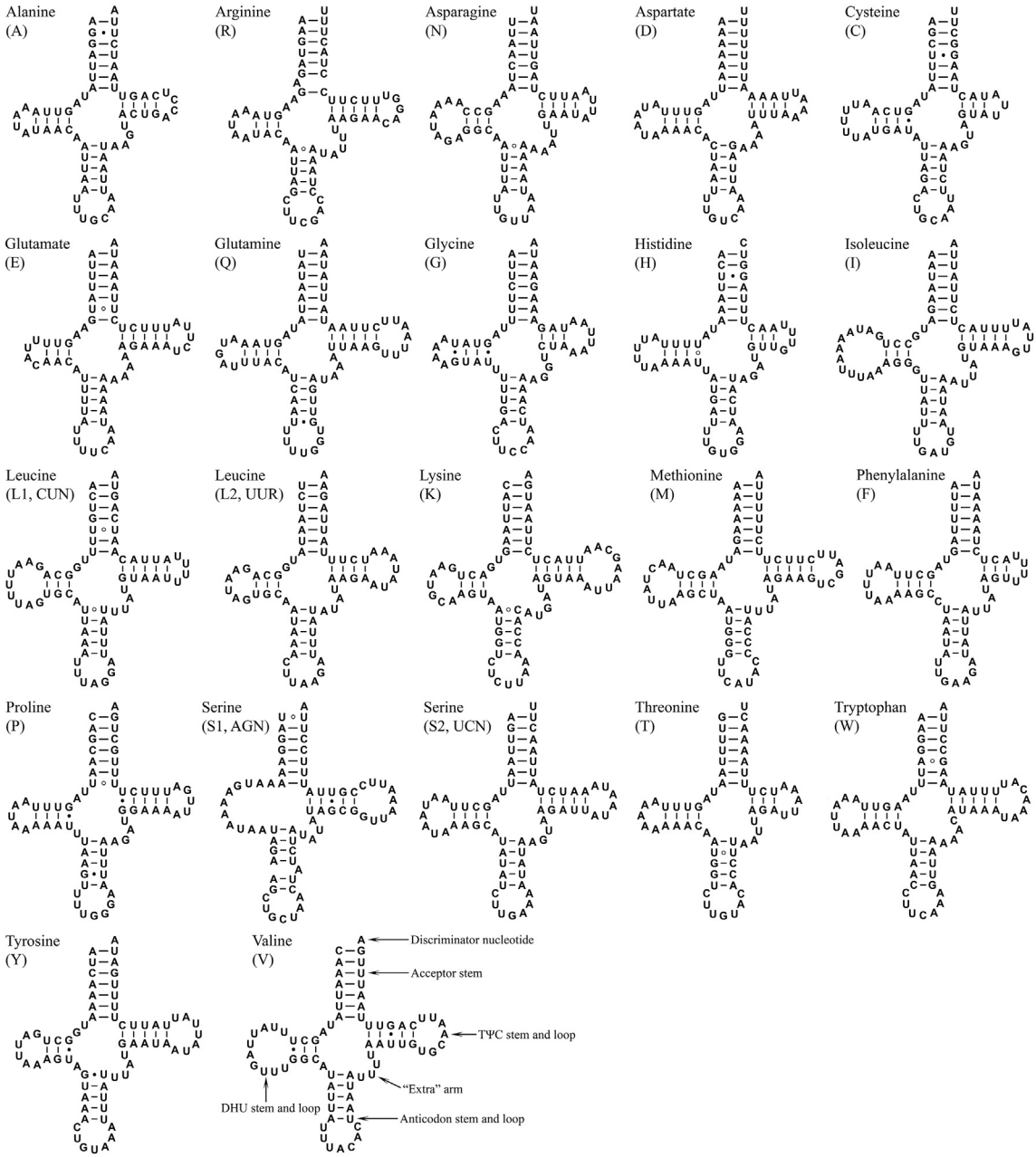

Fig. 3. Secondary structures of tRNAs in the mitogenome of *P. huangshanensis*. The dashes, solid dots, and hollowed dots indicate Watson-Crick bonds, GU pairs, and mismatches, respectively.

only in the whole mitogenome but also in PCGs, rRNAs, and tRNAs. However, slightly negative AT skew and strongly positive GC skew were detected in the control region.

3.2. Protein-coding genes and codon usage

The total length of 13 PCGs of *P. huangshanensis* was 11,072 bp. Most PCGs exhibited the typical start codon ATN (2 ATA, 2 ATT and 7 ATG), while *nad2* and *nad5* initiated with TTG. All PCGs were predicted to use the complete termination codon TAA or TAG, except for *cox2* and *cox3*, which ended with a single T. The truncated stop codons were common in insect mitogenomes and might be converted to TAA by polyadenylation after transcription [39].

Relative synonymous codon usage of *P. huangshanensis* indicated that degenerate codons were biased to use more A/T than G/C in the third codon positions (Fig. 2). For example, the GC-rich codon of GCG-Ala was even absent. Conversely, the four most prevalent codons, including ATT-Ile, ATA-Met, TTA-Leu, and TTT-Phe, were all composed of A and/or T.

3.3. Transfer RNA

The total length of 22 tRNAs was 1476 bp, with the A + T content of 79.3%. Most tRNAs could be folded into the typical clover-leaf secondary structure, whereas *trnS1* was an exception for lacking a dihydrouracil (DHU) arm (Fig. 3). Furthermore, it was found that *trnS1* had an unpaired nucleotide in the anticodon stem, as was the case in the acceptor stem of *trnR*. This unusual feature had also been proposed in the DHU and the anticodon arm of some true bugs [26, 40]. In addition, a total of 23 unmatched base pairs were scattered throughout the tRNAs (the amino acid acceptor (8), DHU (7), anticodon (5), and TΨC stems (3)). Twelve of these were noncanonical matches of G-U pairs, while the

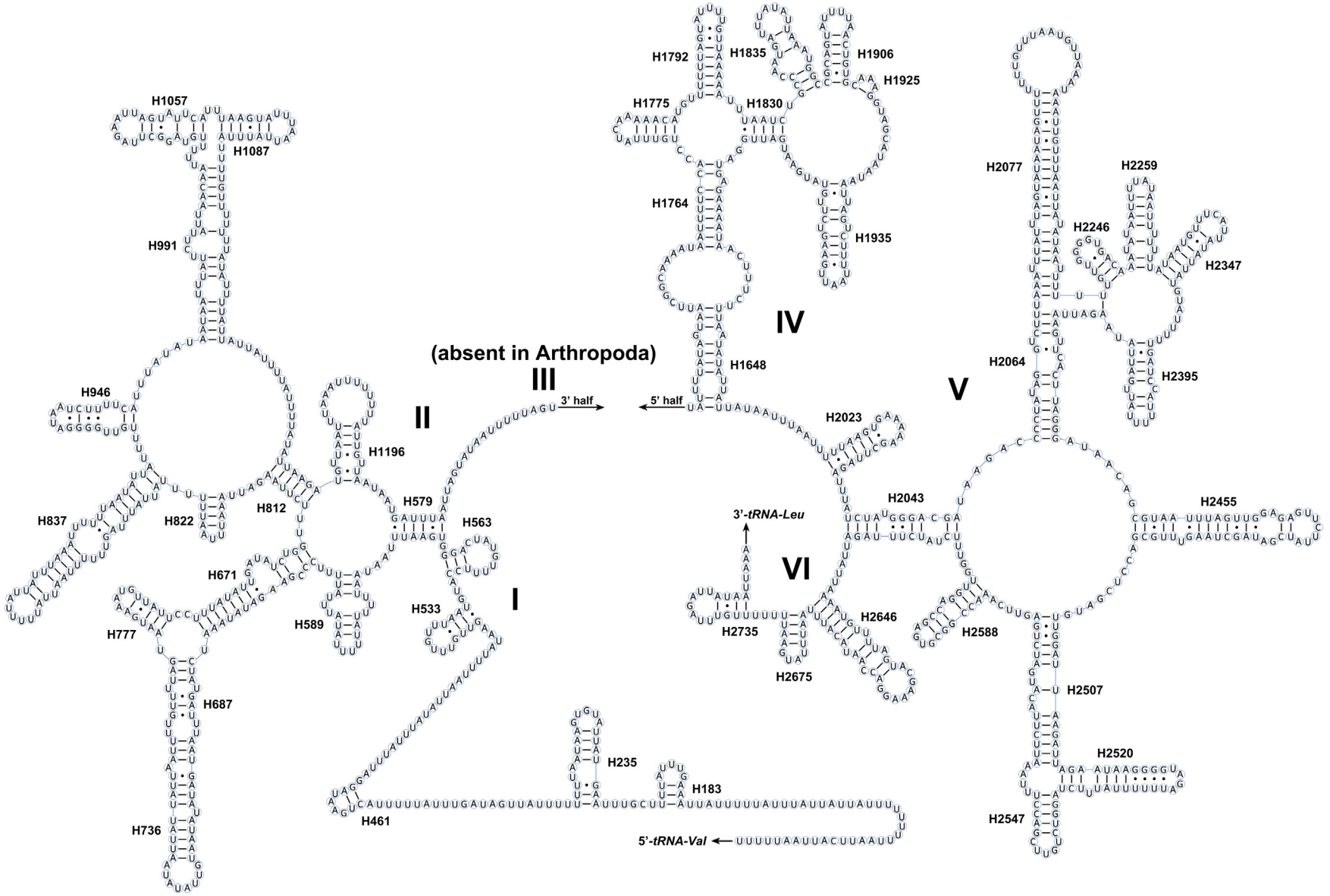

Fig. 4. Predicted secondary structure of the *rrnL* in the mitogenome of *P. huangshanensis*. Roman numerals indicate the conserved domain structure. Watson-Crick pairs are joined by dashes, whereas GU pairs are connected by dots.

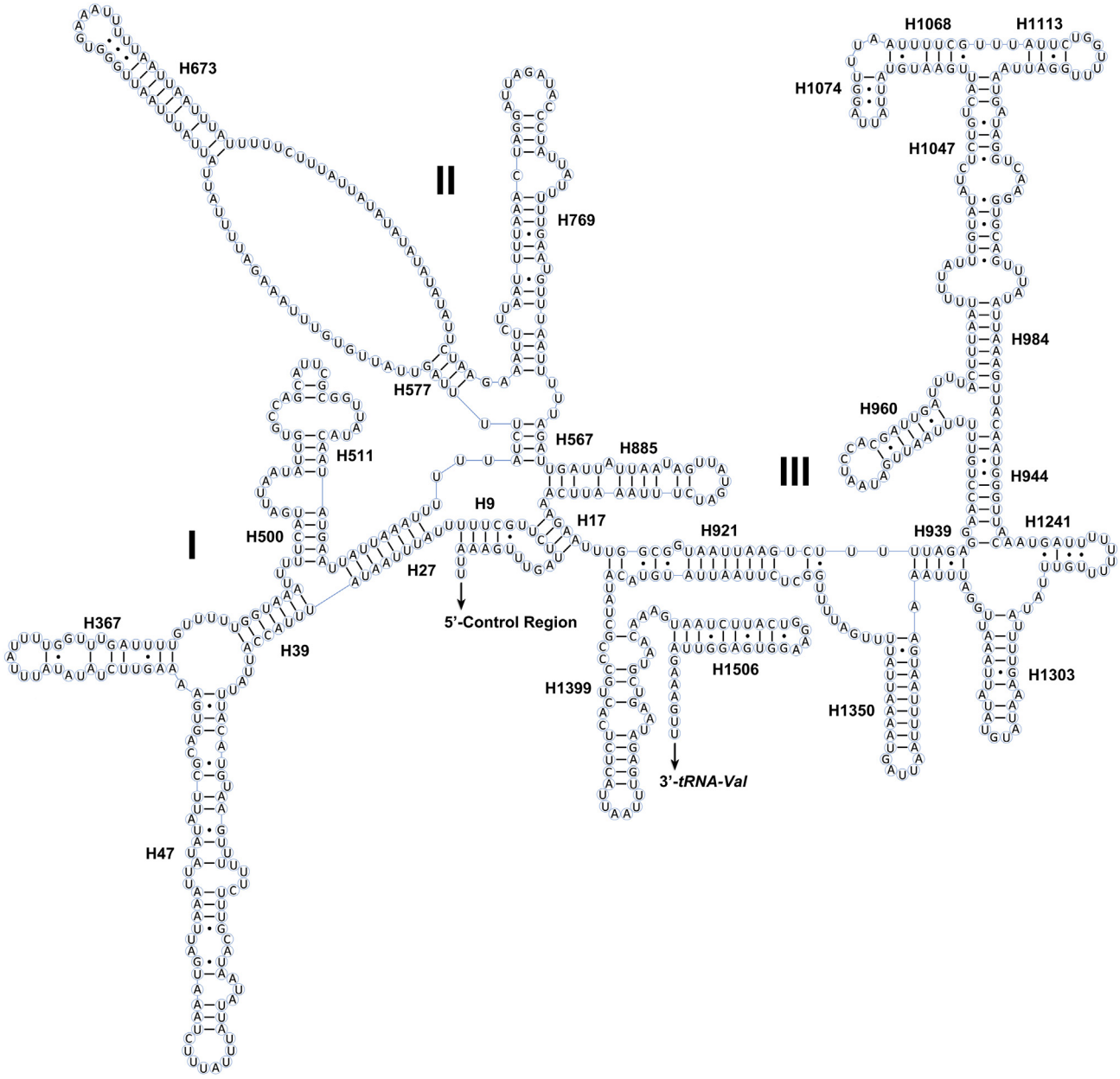

Fig. 5. Predicted secondary structure of the *rrnS* in the mitogenome of *P. huangshanensis*. Roman numerals indicate the conserved domain structure. Watson-Crick pairs are joined by dashes, whereas GU pairs are connected by dots.

remaining 11 mismatches were U-U (7), A-A (2), A-G (1), and A-C (1). The wobble and mismatched pairs were also detected in other Cercopoidea mitogenomes [36].

3.4. Ribosomal RNA

The boundaries of rRNAs were assumed to extend to their flanking genes and were also verified by alignment with the homologous gene sequences. As in other mitogenomes of Cercopoidea [37, 38], both *rrnL* and *rrnS* were encoded on the N-strand (Fig. 1). The *rrnL* gene was located at the position between *trnL1* and *trnV*, with the length of 1269 bp (Table 2). The *rrnS* was assumed to fill up the blank between *trnV* and the control region, with the length of 780 bp. To date, the secondary structures of Cercopoidea *rrnL* and *rrnS* have not been reported. Therefore, in this study, we predicted the *rrnL* and *rrnS* secondary structures of *P. huangshanensis* according to some other hemipterans [23–26].

The secondary structure of *rrnL* consisted of 44 helices and five domains (domain III was absent in insects) (Fig. 4) [28]. Compared with the previously reported *rrnL* structure frameworks of other hemipterans [24–26, 40, 41], domains IV, V, and VI were highly conserved except for helices H1835 and H2455. However, domains I and II were more variable, especially for helices H687 and H991-H1087, which could be folded into different helix structures by the Mfold Web Server. Nevertheless, based on previously published rRNA secondary structures of other hemipterans [25, 26], we chose the one that possessing more paired nucleotide on the stems.

The *rrnS* included 27 Helices and three domains (Fig. 5). Compared to domains I and II, domain III was structurally more conserved except for the region of H1047-H1113. In addition, although H1068 was assumed to be absent in some hemipterans [23, 42], it was presented in the rrnS of *P. huangshanensis*. In domain I, the helices H47 and H367 were highly variable. In fact, no consistent secondary structure had been inferred for these helices in insects [43]. In domain II, although the helix H673 was reported to have a long paired stem and a small

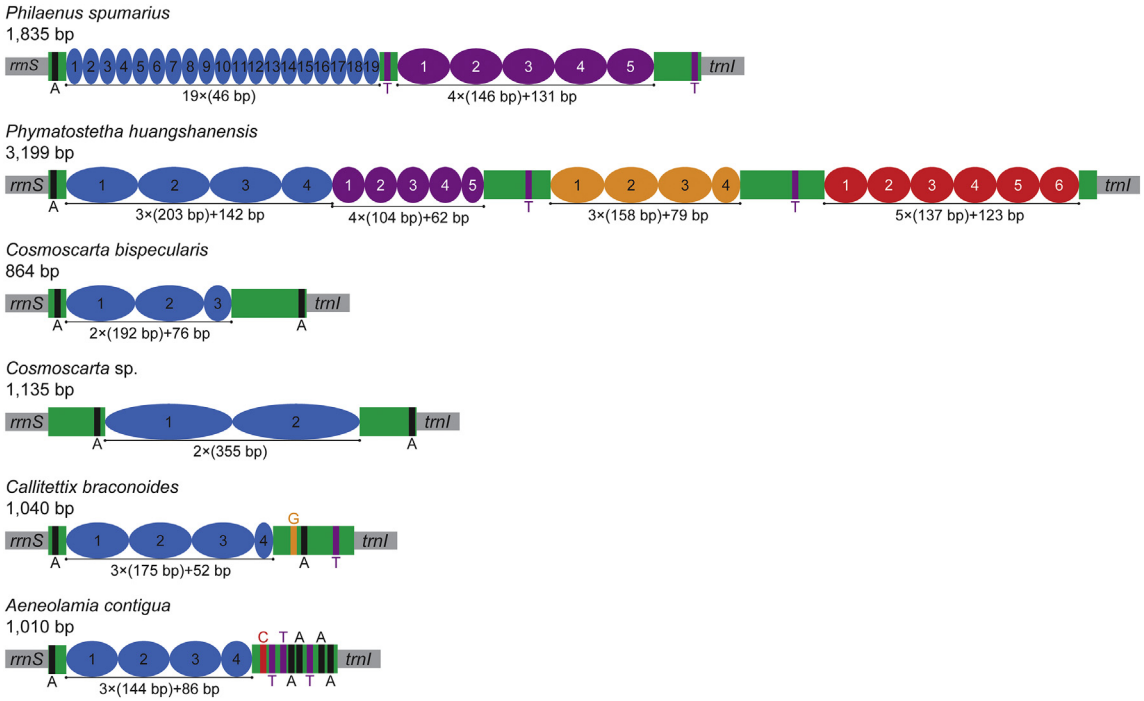

Fig. 6. Organization of the control regions in the Cercopoidea mitogenomes. The location and copy number of tandem repeats are illustrated by colored ovals with Arabic numerals inside. Non-repeat regions are shown by green boxes. The structures of poly(A), poly(T), poly(G), and poly(C) are represented by the black, purple, orange, and red blocks, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

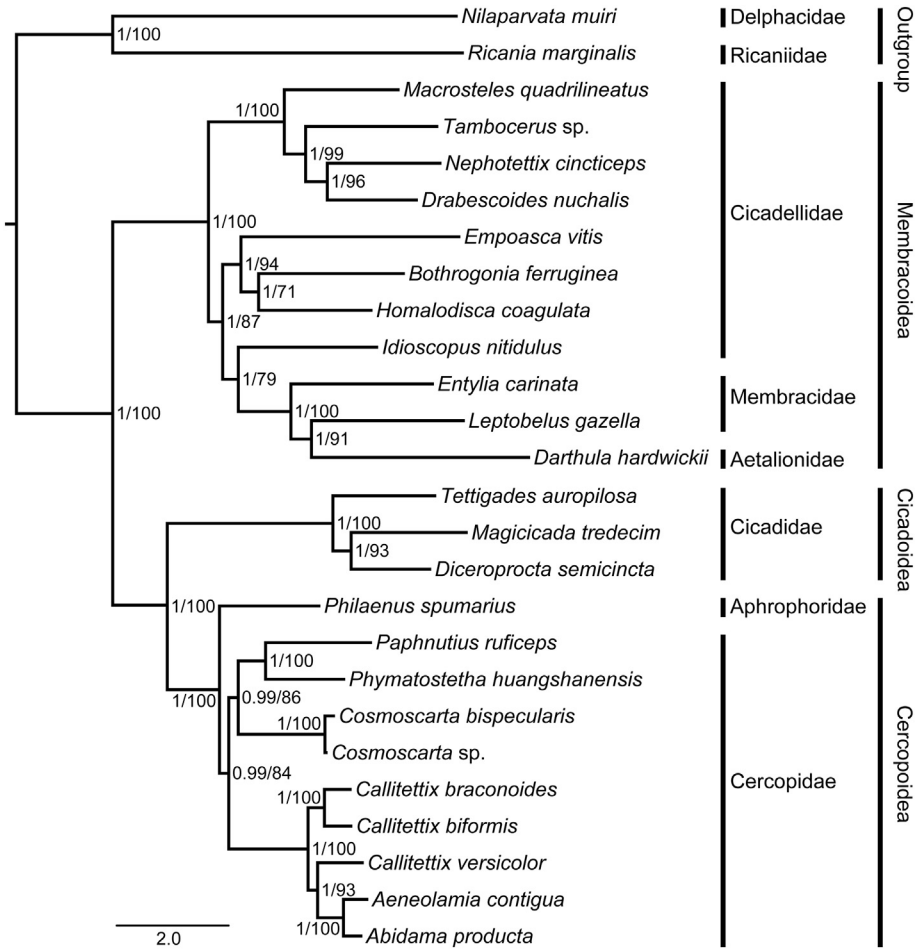

Fig. 7. Phylogenetic relationships inferred from mitogenomes of Cicadomorpha. Numbers at the nodes indicate Bayesian posterior probabilities and ML bootstrap values, respectively.

loop in some insects [24], this region formed a short stem and a very large loop in *P. huangshanensis*, as reported in other hemipterans [26, 44].

3.5. Control region

The control region of *P. huangshanensis* was flanked by *rrnS* and *trnI*, with the high A + T content of 80.9% and the remarkably large size of 3199 bp. Notably, multiple types of tandem repeat elements were detected in the control region of *P. huangshanensis* (Fig. 6; Table S3). Conversely, some Cercopoidea species, including *P. ruficeps*, *Callitettix biformis*, *Callitettix versicolor*, and *Abidama producta*, had no obvious tandem repeats. Control regions of the remaining five Cercopoidea mitogenomes had only 1–2 distinct tandem repeat units. Accordingly, the control region of *P. huangshanensis* was longer than those of other spittlebugs, which ranged from 310 bp in *P. ruficeps* [35] to 1835 bp in *P. spumarius* [36]. The occurrence of tandem repeats was proposed to play a role in DNA methylation or gene transcription [45] and could be added or subtracted during replication through slipped-strand mispairing [46].

Although many Poly(N) sequences, including Poly(A), Poly(T), Poly (G), and Poly(C), were detected in control regions of the tribe Callitettixini (Hemiptera: Cercopoidea: Cercopidae) [37], only 2–3 Poly(A) or Poly(T) could be found in those of other spittlebugs (Fig. 6). Interestingly, a Poly(A) was consistently located at the 5′-end of the control regions in all the sequenced Cercopoidea species.

3.6. Phylogenetic analysis

The phylogenetic analyses were performed on the concatenated nucleotide sequences of 13 PCGs and two rRNAs derived from 24 available Cicadomorpha mitogenomes, with two Fulgoromorpha species as the outgroups. The ML and BI analyses yielded fully resolved trees with the identical topology (Fig. 7). As proposed by previous studies [47–49], the monophyly of each superfamily was robustly supported (BP = 100; PP = 1.00), with the relationships of (Membracoidea + (Cicadoidea + Cercopoidea)).

Within the superfamily Cercopoidea, the monophyly of Cercopidae was also supported. However, the genera *Callitettix* was a paraphyletic group, which was also consistent with recent phylogenetic studies using mitogenomes [37, 50]. The remaining taxa presented the relationships of (*Cosmoscarta* + (*Phymatostetha* + *Paphnutius*)). Our phylogeny clearly demonstrated the identical topology in the two inference methods, but without strong support for ML analysis. We suggest that more thorough sampling will be needed to adequately resolve the phylogenetic relationships within Cercopoidea.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2018.07.135.

Declarations of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [grant numbers 31572298, 31561163003].

References

[1] J.R. Cryan, G.J. Svenson, Family-level relationships of the spittlebugs and froghoppers (Hemiptera: Cicadomorpha: Cercopoidea), Syst. Entomol. 35 (2010) 393–415.
[2] X. Chen, A.P. Liang, Identification of a self-regulatory pheromone system that controls nymph aggregation behavior of rice spittlebug *Callitettix versicolor*, Front. Zool. 12 (2015) 10.
[3] M.R.G.K. Nair, Insects and mites of crops in India, second ed. Indian Council of Agricultural Research, New Delhi, 1975.
[4] B.V. David, Elements of Economic Entomology, Popular Book Depot, Chennai, 2001.
[5] D.X. Zhang, G.M. Hewitt, Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies, Biochem. Syst. Ecol. 25 (1997) 99–120.
[6] J.L. Boore, Animal mitochondrial genomes, Nucleic Acids Res. 27 (1999) 1767–1780.
[7] J.W. Taanman, The mitochondrial genome: structure, transcription, translation and replication, Biochim. Biophys. Acta 1410 (1999) 103–123.
[8] M. Dowton, S.L. Cameron, J.I. Dowavic, A.D. Austin, M.F. Whiting, Characterization of 67 mitochondrial tRNA gene rearrangements in the Hymenoptera suggests that mitochondrial tRNA gene position is selectively neutral, Mol. Biol. Evol. 26 (2009) 1607–1617.
[9] J.L. Boore, D.V. Lavrov, W.M. Brown, Gene translocation links insects and crustaceans, Nature 392 (1998) 667–668.
[10] S.L. Cameron, Insect mitochondrial genomics: implications for evolution and phylogeny, Annu. Rev. Entomol. 59 (2014) 95–117.
[11] J.P. Curole, T.D. Kocher, Mitogenomics: digging deeper with complete mitochondrial genomes, Trends Ecol. Evol. 14 (1999) 394–398.
[12] C.P. Lin, B.N. Danforth, How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets, Mol. Phylogenet. Evol. 30 (2004) 686–702.
[13] J.C. Avise, Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation, Conserv. Biol. 9 (1995) 686–690.
[14] L. Lv, X.X. Peng, S.L. Jing, B.F. Liu, L.L. Zhu, G.C. He, Intraspecific and interspecific variations in the mitochondrial genomes of *Nilaparvata* (Hemiptera: Delphacidae), J. Econ. Entomol. 108 (2015) 2021–2029.
[15] J. Qin, Y.Z. Zhang, X. Zhou, X.B. Kong, S.J. Wei, R.D. Ward, A.B. Zhang, Mitochondrial phylogenomics and genetic relationships of closely related pine moth (Lasiocampidae: Dendrolimus) species in China, using whole mitochondrial genomes, BMC Genomics 16 (2015) 428.
[16] J. Kim, E. Kern, T. Kim, M. Sim, J. Kim, Y. Kim, C. Parke, S.A. Nadler, J.K. Park, Phylogenetic analysis of two *Plectus* mitochondrial genomes (Nematoda: Plectida) supports a sister group relationship between Plectida and Rhabditida within Chromadorea, Mol. Phylogenet. Evol. 107 (2017) 90–102.
[17] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucleic Acids Symp. Ser. 41 (1999) 95–98.
[18] M. Bernt, A. Donath, F. Jühling, F. Externbrink, C. Florentz, G. Fritzsch, J. Pütz, M. Middendorf, P.F. Stadler, MITOS: improved *de novo* metazoan mitochondrial genome annotation, Mol. Phylogenet. Evol. 69 (2013) 313–319.
[19] J.R. Grant, A.S. Arantes, P. Stothard, Comparing thousands of circular genomes using the CGView Comparison Tool, BMC Genomics 13 (2012) 202.
[20] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA 6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (2013) 2725–2729.
[21] N.T. Perna, T.D. Kocher, Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes, J. Mol. Evol. 41 (1995) 353–358.
[22] G. Benson, Tandem repeats finder: a program to analyze DNA sequences, Nucleic Acids Res. 27 (1999) 573–580.
[23] Y. Wang, X.L. Huang, G.X. Qiao, Comparative analysis of mitochondrial genomes of five aphid species (Hemiptera: aphididae) and phylogenetic implications, PLoS One 8 (2013), e77511.
[24] M.L. Yuan, Q.L. Zhang, Z.L. Guo, J. Wang, Y.Y. Shen, Comparative mitogenomic analysis of the superfamily Pentatomoidea (Insecta: Hemiptera: Heteroptera) and phylogenetic implications, BMC Genomics 16 (2015) 460.
[25] Z.T. Chen, L.X. Mu, J.R. Wang, Y.Z. Du, Complete mitochondrial genome of the citrus spiny whitefly *Aleurocanthus spiniferus* (Quaintance) (Hemiptera: Aleyrodidae): implications for the phylogeny of whiteflies, PLoS One 11 (2016), e0161385.
[26] T. Li, J. Yang, Y.W. Li, Y. Cui, Q. Xie, W.J. Bu, D.M. Hillis, A mitochondrial genome of Rhyparochromidae (Hemiptera: Heteroptera) and a comparative analysis of related mitochondrial genomes, Sci. Rep. 6 (2016) 35175.
[27] M. Zuker, Mfold web server for nucleic acid folding and hybridization prediction, Nucleic Acids Res. 31 (2003) 3406–3415.
[28] J.J. Cannone, S. Subramanian, M.N. Schnare, J.R. Collett, L.M. D'Souza, Y. Du, B. Feng, N. Lin, L.V. Madabusi, K.M. Müller, N. Pande, Z. Shang, N. Yu, R.R. Gutell, The Comparative RNA Web (CRW) Site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs, BMC Bioinf. 3 (2002) 2.
[29] K. Katoh, D.M. Standley, MAFFT multiple sequence alignment so ware version 7: improvements in performance and usability, Mol. Biol. Evol. 30 (2013) 772–780.
[30] J. Castresana, Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis, Mol. Biol. Evol. 17 (2000) 540–552.
[31] R. Lanfear, P.B. Frandsen, A.M. Wright, T. Senfeld, B. Calcott, PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses, Mol. Biol. Evol. 34 (2016) 772–773.
[32] L.T. Nguyen, H.A. Schmidt, A. von Haeseler, B.Q. Minh, IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies, Mol. Biol. Evol. 32 (2015) 268–274.
[33] F. Ronquist, M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Hohna, B. Larget, L. Liu, M.A. Suchard, J.P. Huelsenbeck, MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space, Syst. Biol. 61 (2012) 539–542.
[34] M.A. Miller, W. Pfeiffer, T. Schwartz, Creating the CIPRES Science Gateway for inference of large phylogenetic trees, Gateway Computing Environments Workshop (GCE), Gateway Computing Environments Workshop (GCE), vol. 14, 2010, pp. 1–8.
[35] J. Liu, A.P. Liang, The complete mitochondrial genome of spittlebug *Paphnutius ruficeps* (Insecta: Hemiptera: Cercopidae) with a fairly short putative control region, Acta Biochim. Biophys. Sin. 45 (2013) 309.
[36] J.B. Stewart, A.T. Beckenbach, Insect mitochondrial genomics: the complete mitochondrial genome sequence of the meadow spittlebug *Philaenus spumarius* (Hemiptera: Auchenorrhyncha: Cercopoidae), Genome 48 (2005) 46–54.

[37] J. Liu, C.P. Bu, B. Wipfler, A.P. Liang, Comparative analysis of the mitochondrial genomes of Callitettixini spittlebugs (Hemiptera: Cercopidae) confirms the overall high evolutionary speed of the AT-rich region but reveals the presence of short conservative elements at the tribal level, PLoS One 9 (2014), e109140.
[38] H. Yang, J. Liu, A.P. Liang, The complete mitochondrial genome of *Cosmoscarata bispecularis* (Hemiptera, Cicadomorpha, Cercopoidea, Cercopidae), Mitochondrial DNA Part A 27 (2016) 3957–3958.
[39] D. Ojala, J. Montoya, G. Attardi, tRNA punctuation model of RNA processing in human mitochondrial, Nature 290 (1981) 470–474.
[40] P. Wang, H. Li, Y. Wang, J.H. Zhang, X. Dai, J. Chang, B.W. Hu, W.Z. Cai, The mitochondrial genome of the plant bug *Apolygus lucorum* (Hemiptera: Miridae): presently known as the smallest in Heteroptera, Insect Sci. 21 (2014) 159–173.
[41] F. Yu, A.P. Liang, The complete mitochondrial genome of *Ugyops* sp. (Hemiptera: Delphacidae), J. Insect Sci. 18 (2018) 25.
[42] H. Li, H. Liu, A.M. Shi, P. Štys, X.G. Zhou, W.Z. Cai, The complete mitochondrial genome and novel gene arrangement of the unique-headed bug *Stenopirates* sp. (Hemiptera: Enicocephalidae), PLoS One 7 (2012), e29419.
[43] S.L. Cameron, M.F. Whiting, The complete mitochondrial genome of the tobacco hornworm, *Manduca sexta*, (Insecta: Lepidoptera: Sphingidae), and an examination of mitochondrial gene variability within butterflies and moths, Gene 408 (2008) 112–123.
[44] J.Y. Gao, H. Li, X.L. Truong, X. Dai, J. Chang, W.Z. Cai, Complete nucleotide sequence and organization of the mitochondrial genome of *Sirthenea flavipes* (Hemiptera: Reduviidae: Peiratinae) and comparison with other assassin bugs, Zootaxa 3669 (2013) 1–16.
[45] W. Huang, J. Zheng, Y. He, C. Luo, Tandem repeat modification during double-strand break repair induced by an engineered TAL effector nuclease in zebrafish genome, PLoS One 8 (2013), e84176.
[46] G. Levinson, G.A. Gutman, Slipped-strand mispairing: a major mechanism for DNA sequence evolution, Mol. Biol. Evol. 4 (1987) 203–221.
[47] J.R. Cryan, Molecular phylogeny of Cicadomorpha (Insecta: Hemiptera: Cicadoidea, Cercopoidea and Membracoidea): adding evidence to the controversy, Syst. Entomol. 30 (2005) 563–574.
[48] J.R. Cryan, J.M. Urban, Higher-level phylogeny of the insect order Hemiptera: is Auchenorrhyncha really paraphyletic? Syst. Entomol. 37 (2012) 7–21.
[49] H. Li, J.M. Leavengood Jr., E.G. Chapman, D. Burkhardt, F. Song, P. Jiang, J.P. Liu, X.G. Zhou, W.Z. Cai, Mitochondrial phylogenomics of Hemiptera reveals adaptive innovations driving the diversification of true bugs, Proc. R. Soc. B 284 (2017) 20171223.
[50] N. Song, W.Z. Cai, H. Li, Deep-level phylogeny of Cicadomorpha inferred from mitochondrial genomes sequenced by NGS, Sci. Rep. 7 (2017) 10429.