The Complete Mitogenome of Curculio Chinensis (Coleoptera: Curculionidae: Curculioninae): Structural Characterization and Phylogenetic Implication

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Abstract

To explore the phylogenetic position of *Curculio chinensis* Chevrolat, 1878 and phylogenetic relationships among major lineages of the family Curculionidae, we sequenced and annotated this mitogenome. The mitogenome is 18,680 bp in length, and includes the 37 typical mitochondrial genes and a large control region (length: 1,997 bp). Mitogenome organization, nucleotide composition, and codon usage are similar to most of the previously sequenced Curculioninae mitogenomes. All 13 protein-coding genes use ATN or TTG as start codon, and end with TAA/G or incomplete stop codons (single T-). Twenty-one transfer RNA genes have the typical clover-leaf structures, while the dihydrouridine (DHU) arm of *trnS1* is missing. In Curculioninae mitogenomes, the size and number of tandem repeats in the control region are highly variable. Both ML and BI analyses based on the 13 PCGs and two rRNAs from 91 species of Coleoptera strongly supported the monophyly of Curculionidae and three of the included subfamilies (Platypodinae, Dryophthorinae, and Cryptorhynchinae) plus the sister relationship between Platypodinae and Dryophthorinae. Additionally, the monophyly of the genus *Curculio* was recovered with strong support.

1. Introduction

The typical mitogenome of insects is a circular double-stranded DNA molecule with 15–18 kb in length, encoding 13 protein-coding genes (PCG), two ribosomal RNA genes (rRNA) and 22 transfer RNA genes (tRNA), also includes a large non-coding region (control region) [1, 2]. In insects, the mitogenome has been widely used as a molecular marker to explore the population genetics, phylogeny, and evolution [2–4].

The camellia weevil, *Curculio chinensis* Chevrolat, 1878, belongs to the subfamily Curculioninae (Coleoptera: Curculionidae), and is widely distributed in most of China's *Camellia* spp. (tea) producing areas [5]. It is one of the most serious pests of tea and causes huge economic losses [5, 6]. Further understanding of the phylogenetic status of *C. chinensis* is of great interest to the management of economic plant pests.

In this study, we sequenced and annotated the mitogenome of *C. chinensis*, and analyzed its characteristics. Phylogenetic relationships were reconstructed based on nucleotide sequence data of 91 species mitogenomes, which enabled us to investigate the phylogenetic position of *C. chinensis* and provided insight into the phylogenetic relationships among the major subfamilies of Curculionidae.

2. Materials And Methods

2.1 Sample collection and DNA extraction

Adult specimens of *C. chinensis* were collected from Yunguanshan forest farm, Guiyang City, Guizhou Province, China (26.48208727° N, 106.75480714° E, July 2020). All fresh specimens were preserved in 100% ethyl alcohol and deposited in a -20℃ freezer at the laboratory of Guizhou Academy of Forestry,

Guiyang. Identification specimens was performed using morphological characters in Chao and Chen [7]. Whole genomic DNA was extracted from thorax muscle tissues using the Biospin Insect Genomic DNA Extraction Kit (BioFlux) following manufacturer's instructions. Voucher specimens are stored in the insect herbarium of Guizhou Academy of Forestry.

2.2 Mitogenome sequencing, assembly, annotation, and bioinformatic analyses

The complete mitogenome of *C. chinensis* was sequenced using NGS (next-generation sequencing) (Illumina HiSeq X10; Biomarker Technologies Corporation, Beijing, China). About 1.26 Gb clean data was assembled into a complete circular mitogenome by NOVOPlasty v2.7.0 [8] using the *cox1* sequence of *Curculio davidi* (Curculionidae: Curculioninae: GenBank accession: NC_034293) [9] as an initial seed. Mitogenome was annotated by MITOZ v1.04 [10] and checked manually in Geneious 8.1.3 (Biomatters, Auckland, New Zealand). tRNA secondary structures were manually drawn using Adobe Illustrator CC2017 based on MITOS Web Server [11] predictions. Mitogenome map was drawn with the program Organellar Genome DRAW (OGDRAW) [12]. Tandem repeat units within the control region were identified in tandem repeats finder [13]. Bioinformatic analyses, including nucleotide composition, composition skew, codon usage of PCGs, relative synonymous codon usage (RSCU), and mitogenomic organization tables were conducted using PhyloSuite v1.2.2 [14].

2.3 Molecular phylogenetic analysis

A total of 91 mitogenomes from three families of Coleoptera were used for the phylogenetic analyses (Table S1). Of these, eighty-seven species belong to Curculionidae (the ingroup), while the remaining four species from two families (Anthribidae and Brentidae) were chosen as outgroups. Nucleotide sequences (without stop codons) for the 13 PCGs were aligned using MAFFT 7 [15] with the G-INS-i (accurate) strategy and codon alignment mode (Code table: Invertebrate mitochondrial genetic codon). rRNAs genes (*rrnL* and *rrnS*) were aligned using the MAFFT 7 [15] with the Q-INS-I algorithm (which takes account of the secondary structure of rRNA gene). Ambiguously aligned areas were removed using Gblocks v0.91b [16], respectively. Gene alignments were concatenated using PhyloSuite v1.2.2 [14]. Partitioning scheme and nucleotide substitution models for maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses were selected with PartitionFinder2 [17] using the Bayesian information criterion (BIC) (Tables S2-S3). ML analyses were reconstructed by IQ-TREE [18] under the ultrafast bootstrap (UFB) approximation approach [19] with 10,000 replicates. BI analyses were performed using MrBayes 3.2.6 [20] in the CIPRES Science Gateway [21] with four chains (one cold chain and three hot chains). Two independent runs of 30,000,000 generations were carried out with sampling every 1,000 generations. The first 25% of trees were discarded as burn-in. After the average standard deviation of split frequencies fell below 0.01, stationarity was assumed.

3. Results And Discussion

3.1 Mitogenome organization and nucleotide composition

The mitogenome of *C. chinensis* is a double-stranded circular DNA molecule, containing 37 typical mitochondrial genes (13 PCGs, 22 tRNAs, and two rRNAs) and a large control region (Table 1, Fig. 1), which are common in bilaterian animals [2]. The newly sequenced mitogenome (length: 18,680 bp) is medium-sized compared to other Curculioninae mitogenomes (ranging from 16,852 bp *Curculio*. *davidi*, GenBank accession: NC_034293 to 19,216 bp *Curculio* sp. GenBank accession: MG728095) [9]. Variation in the size of the control region is the main source of the length variation in Curculioninae mitogenomes. The mitogenome of *C. chinensis* has the same gene order as other previously sequenced Curculioninae species [9, 22, 23]. A total of 71 overlapping nucleotides were found in ten pairs of neighboring genes, the longest overlap (23 bp) was identified between the *trnL1* and *rrnL*. Furthermore, there are 2,033 intergenic nucleotides disperse across 14 gene boundaries, and the longest intergenic region (1,882 bp) is located between *trnI* and *trnQ*.

Table 1
Mitogenomic organization of *C. chinensis*.

Name	Location		Size (bp)	Intergenic nucleotides	Codon		Strand
	From	To			Start	Stop	
trnI	1	65	65				+
trnQ	1948	2016	69	1882			-
trnM	2018	2085	68	1			+
nad2	2089	3096	1008	3	ATA	TAA	+
trnW	3111	3174	64	14			+
trnC	3174	3239	66	-1			-
trnY	3242	3305	64	2			-
cox1	3298	4842	1545	-8	ATT	TAA	+
trnL2	4838	4902	65	-5			+
cox2	4903	5586	684		ATT	TAA	+
trnK	5588	5658	71	1			+
trnD	5661	5725	65	2			+
atp8	5726	5884	159		ATT	TAA	+
atp6	5881	6552	672	-4	ATA	TAA	+
cox3	6563	7343	781	10	ATT	T	+
trnG	7344	7407	64				+
nad3	7408	7761	354		ATT	TAG	+
trnA	7760	7826	67	-2			+
trnR	7827	7888	62				+
trnN	7887	7950	64	-2			+
trnS1	7951	8017	67				+
trnE	8025	8088	64	7			+
trnF	8089	8153	65				-
nad5	8137	9873	1737	-17	ATT	TAA	-
trnH	9874	9936	63				-
nad4	9937	11272	1336		ATG	T	-

Name	Location		Size (bp)	Intergenic nucleotides	Codon		Strand
	From	To			Start	Stop	
nad4L	11266	11559	294	-7	ATG	TAA	-
trnT	11562	11626	65	2			+
trnP	11627	11692	66				-
nad6	11695	12198	504	2	ATT	TAA	+
cob	12202	13338	1137	3	ATA	TAA	+
trnS2	13339	13405	67				+
nad1	13509	14459	951	103	TTG	TAG	-
trnL1	14461	14525	65	1			-
rrnL	14503	15831	1329	-23			-
trnV	15830	15895	66	-2			-
rrnS	15896	16683	788				-
Control region	16684	18680	1997				+

The nucleotide content of the Curculioninae mitogenomes exhibits strong AT bias: (72.5%-77.5%) in the whole genome, (71%-76.1%) in the PCGs, (74.6%-78.3%) in the tRNAs, (76.4%-78.8%) in the rRNAs, and (73.9%-85.6%) in the control region (Tables S4-S12). In almost all sequenced mitogenome of Curculioninae, PCGs have the lowest AT content, while the control region has the highest AT content (except for *Anthonomus eugenii*, *A. rectirostris*, *A. pomorum*, and *Elaeidobius kamerunicus*) [22, 23]. All nine Curculioninae mitogenomes have positive AT-skews (0.04–0.085) and negative GC-skews (– 0.17 to – 0.224), similar to other recently reported weevil mitogenomes [23–26] and most other insects [27].

3.2 Protein-coding genes

The total size of all 13 PCGs of *C. chinensis* is 11,160 bp, accounting for 59.74% of the entire mitogenome (Table 1). In 13 PCGs, *nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad5*, *nad4*, *nad4L*, *nad6*, and *cob* use ATN (ATA/T/G/C) as the start codon, while *nad1* is initiated by TTG, which is common for Curculioninae mitogenomes [9, 22, 23]. All PCGs stopped with TAA/G, or their incomplete form single T-. The incomplete termination codon single T- can be completed by post-transcriptional polyadenylation [28]. The AT-skews of the all PCGs among Curculioninae range from – 0.13 (*A. rectirostris* and *E. kamerunicus*) [22, 23] to -0.146 (*C. davidi*) [9], showing a biased use for the T nucleotide. The relative synonymous codon usage (RSCU) of *C. chinensis* mitogenome is presented in Fig. 2, indicating Leu, Phe, and Ile are the three most frequently used amino acids. In the new mitogenome, the four most frequently utilized codons are UUA-Leu, UUU-Phe, AUU-Ile, and AUA-Met. The most frequently used codons are composed of A nucleotide or U nucleotide, which reflects the high AT content of PCGs.

3.3. Transfer and ribosomal RNA genes

The typical sets of 22 tRNAs were identified with the sizes ranging from 62 bp (*trnR*) to 71 bp (*trnK*) (Table 1). The AT content of tRNAs (74.6%-78.3%) was slightly higher than that of the PCGs (72.5%-77.5%) (Tables S4-S12). Most tRNAs have clover-leaf secondary structures, except for *trnS1*, where the dihydrouridine (DHU) arm became a simple loop (Fig. 3). This feature is common in metazoan mitogenomes [29]. There are a total of 30 mismatched base pairs belong to six types (U-G, U-U, A-C, A-G, U-C, and A-A) were found in the arm structures of the 22 tRNAs.

The length of *rrnS* and *rrnL* genes ranges from 2,043 bp (*E. kamerunicus*) [23] to 2,117 bp (*C. chinensis*), and AT content of rRNAs is conserved in the Curculioninae (Tables S4-S12). For *C. chinensis*, the *rrnL* gene (length: 1329 bp) is encoded between *trnL1* and *trnV*, and the *rrnS* gene (length: 788 bp) is encoded between *trnV* and the control region, similar to other sequenced Curculioninae [9, 22, 23].

3.4 Control region

The control region regulates the replication and transcription of mtDNA [1, 2]. In all sequenced Curculioninae mitogenomes, the control regions are located between *rrnS* and *trnI*. Tandem repeats finder analysis [13] found different numbers of tandem repeat units in the nine complete Curculioninae mitogenomes (Fig. 4). Two types of tandem repeats were discovered in the *A. pomorum* control region (length: 1508 bp; nucleotide positions: 838 to 877 and 1,296 to 1,354). The control region of *A. rectirostris* (length: 1505 bp), *C. chinensis* (length: 1997 bp), *C. davidi* (length: 2138 bp), *Curculio elephas* (length: 2128 bp), *Curculio* sp. (length: 2360 bp), and *E. kamerunicus* (length: 2588 bp) each have one kind of tandem repeat, at positions 461 to 528, 403 to 909, 489 to 701, 242 to 637, 621 to 760, and 486 to 719, respectively. However, in *A. eugenii* and *Anthonomus rubi* control regions, no tandem repeat units were identified. Tandem repeats are believed to be involved in the control of DNA methylation, gene transcription and replication [30, 31].

3.5 Phylogenetic relationships

Based on ML and BI analyses of nucleotide data of 13 PCGs and two rRNAs, we reconstructed the phylogenetic relationships of 91 species of Coleoptera. The trees of two analyses have largely congruent topologies, with most branches strongly supported (Figs. 5–6). Furthermore, relationships recovered in our analyses are similar to those found by Song et al. [24]. As in recent phylogenetic analyses of Curculionidae [32, 33], deep internal nodes within the family are not consistent and received weak support. For instance, in the BI tree (Fig. 6), Cryptorhynchinae is sister to the clade comprising Ceutorhynchinae, Curculioninae, Molytinae, and Alcidinae, while in the ML tree (Fig. 5), Cryptorhynchinae, Molytinae, Ceutorhynchinae, and part of Curculioninae (*E. kamerunicus*, *C. chinensis*, *C. sp.*, *C. davidi*, and *C. elephas*) cluster a clade sister to the remaining Curculioninae (*A. eugenii*, *A. rubi*, *A. rectirostris*, *A. pomorum*, and *Bradybatus kellneri*). The clade comprising Platypodinae and Dryophthorinae is sister to the other Curculionidae both in ML and BI trees (BS = 100; PP = 1.00), consistent with previous studies [24, 33].

All analyses consistently support the monophyly of Curculionidae and some included subfamilies (Platypodinae, Dryophthorinae, and Cryptorhynchinae) with strong support (BS > 96; PP = 1.00). Relationships among Platypodinae, Dryophthorinae, Entiminae, Cyclominae, Scolytinae, and a clade comprising Ceutorhynchinae, Curculioninae, Molytinae, and Alcidinae, were largely congruent across both ML and BI (Figs. 5–6), and the support of most branches is high (BS > 92; PP = 1.00). Within Curculioninae, the monophyly of the genus *Curculio* was recovered with strong support (BS = 100; PP = 1.00), in agreement with previous studies [24, 32].

4. Conclusion

We have sequenced, annotated, and analyzed the mitogenome of *C. chinensis*, whose organization, nucleotide composition, and RSCU are similar to those of other previously sequenced Curculioninae. The phylogenetic analyses based on the nucleotide data of 13 PCGs and two rRNAs of 91 species from Coleoptera strongly supported the monophyly of Curculionidae and three of the included subfamilies (Platypodinae, Dryophthorinae, and Cryptorhynchinae) plus the sister relationship between Platypodinae and Dryophthorinae. Additionally, the monophyly of the genus *Curculio* was recovered with strong support.

Declarations

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Author contributions The study was conceptualised by Zaihua Yang and Niannian Zhang organised the sample collection. Kai Hu conducted all the laboratory work. Kai Hu and Zaihua Yang have written the manuscript.

Compliance with ethical standards

Conflicts of interest The authors declare there are no competing interests.

Consent to Participate The authors provide consent to participate this work.

Consent for publication The authors provide consent to publish this manuscript.

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Tables

Table 1. Mitogenomic organization of *C. chinensis*.

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trnK	5588	5658	71	1			+
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trnR	7827	7888	62				+
trnN	7887	7950	64	-2			+
trnS1	7951	8017	67				+
trnE	8025	8088	64	7			+
trnF	8089	8153	65				-
nad5	8137	9873	1737	-17	ATT	TAA	-
trnH	9874	9936	63				-

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trnP	11627	11692	66				-
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cob	12202	13338	1137	3	ATA	TAA	+
trnS2	13339	13405	67				+
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trnL1	14461	14525	65	1			-
rrnL	14503	15831	1329	-23			-
trnV	15830	15895	66	-2			-
rrnS	15896	16683	788				-
Control region	16684	18680	1997				+

Figures

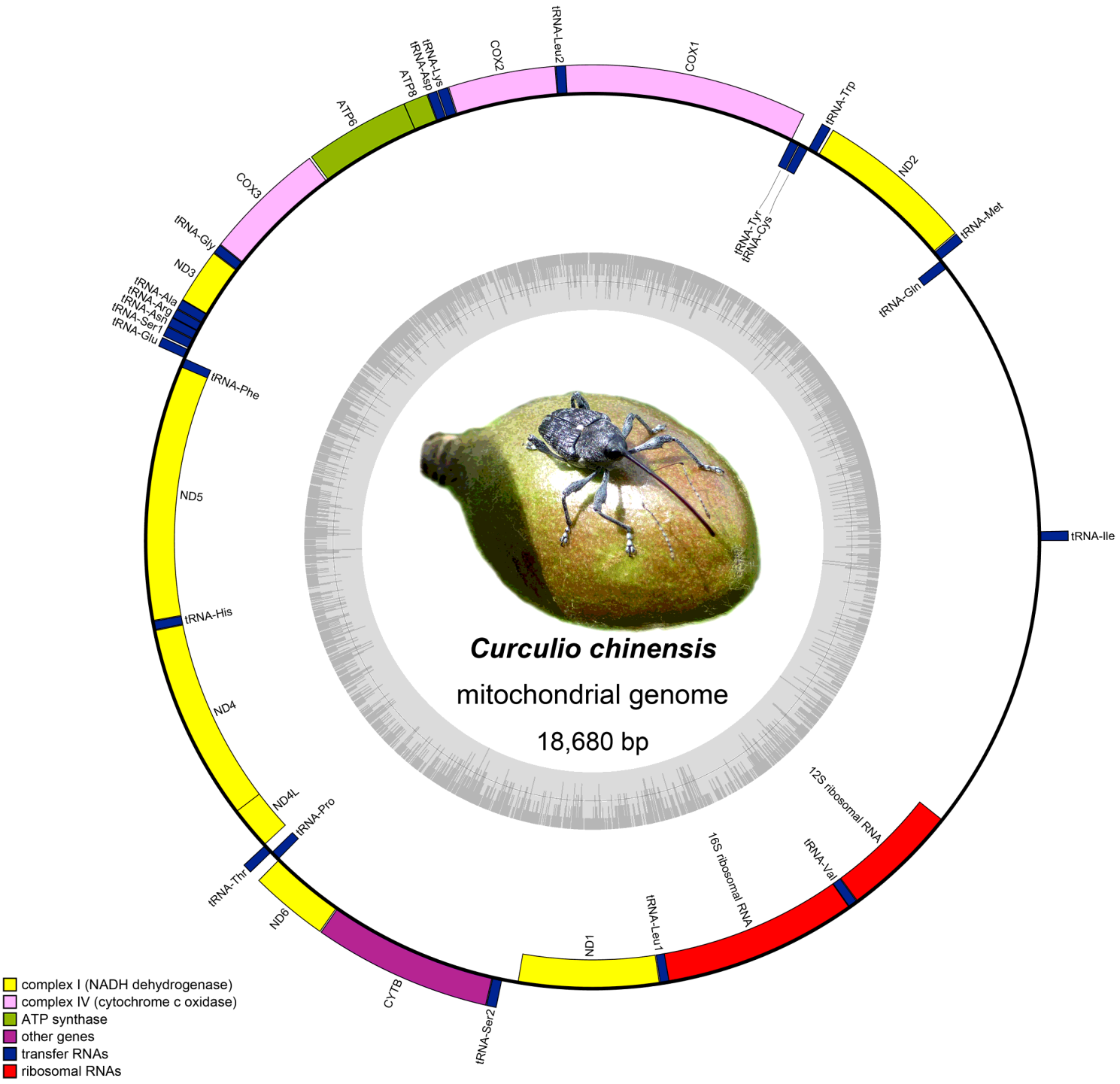

Figure 1

Circular map of the mitogenome of C. chinensis.

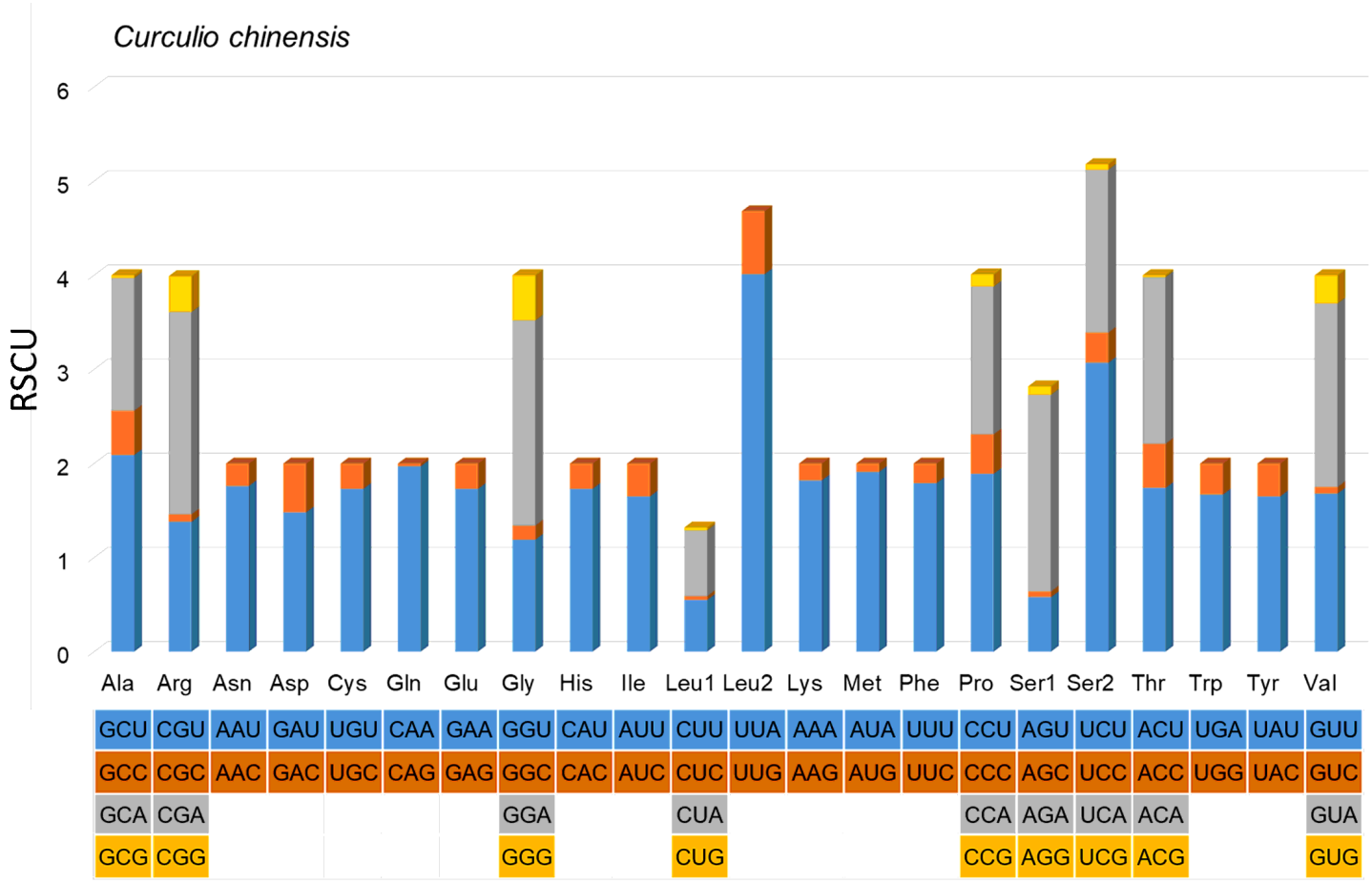

Figure 2

Relative synonymous codon usage (RSCU) of the mitogenome of C. chinensis. The stop codon is not shown.

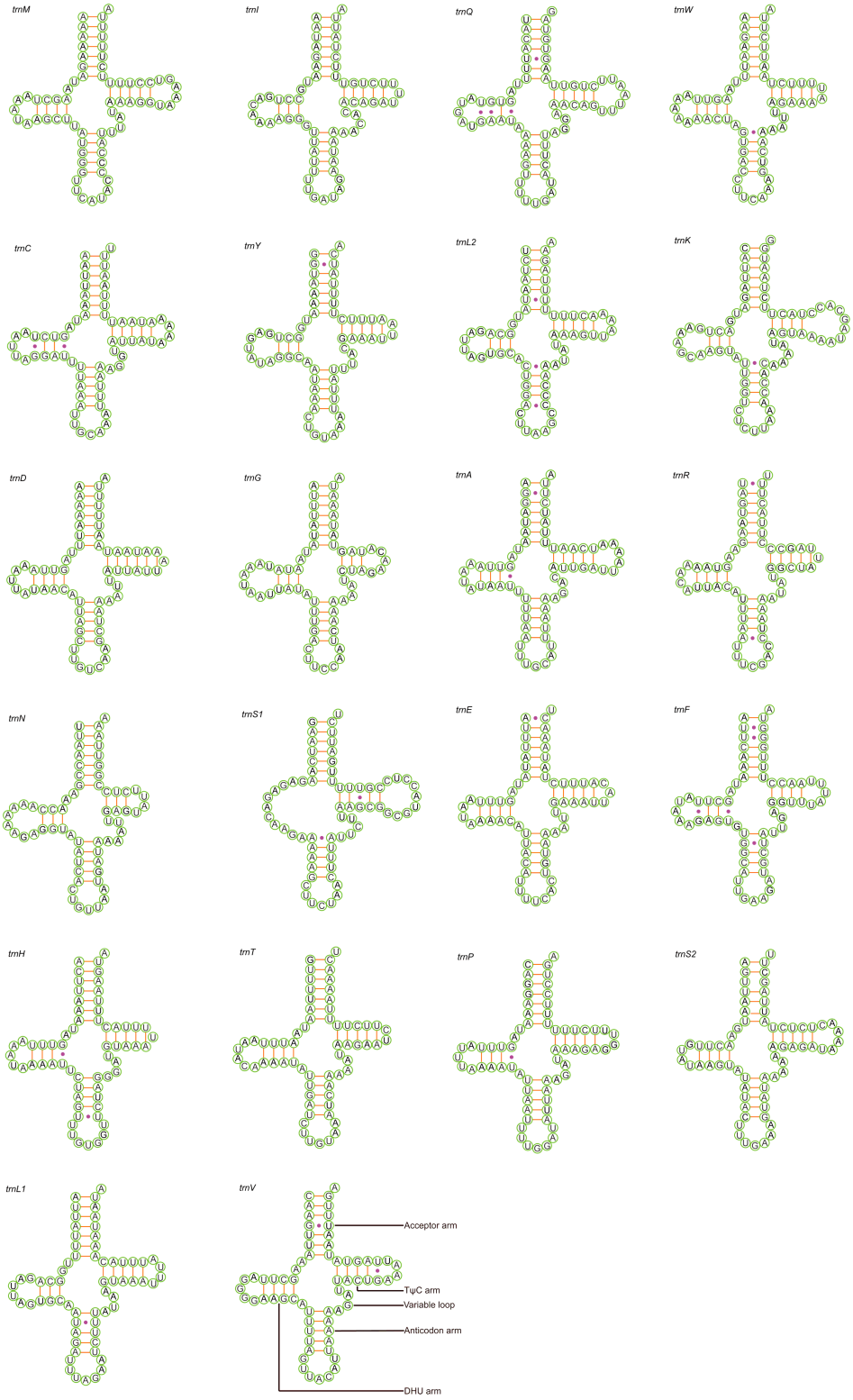

Figure 3

Secondary structures of 22 tRNAs in the mitogenome of C. chinensis. Lines (-) indicate Watson-Crick base pairings, whereas dots (·) indicate unmatched base pairings.

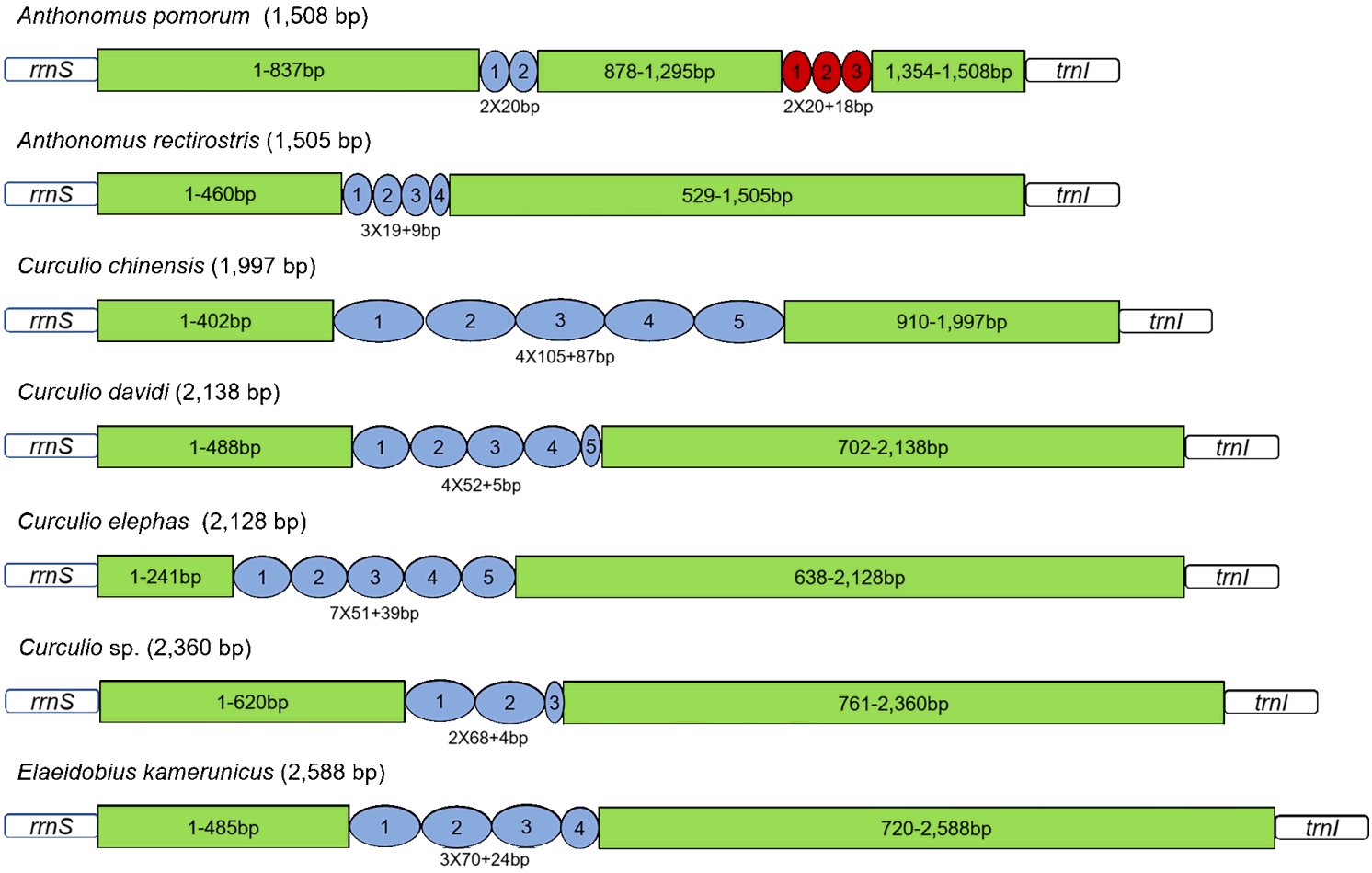

Figure 4

Structures of control regions in the seven complete Curculioninae mitogenomes. The location and copy number of tandem repeat units are displayed by blue and red ovals. Green boxes indicate non-repeat regions.

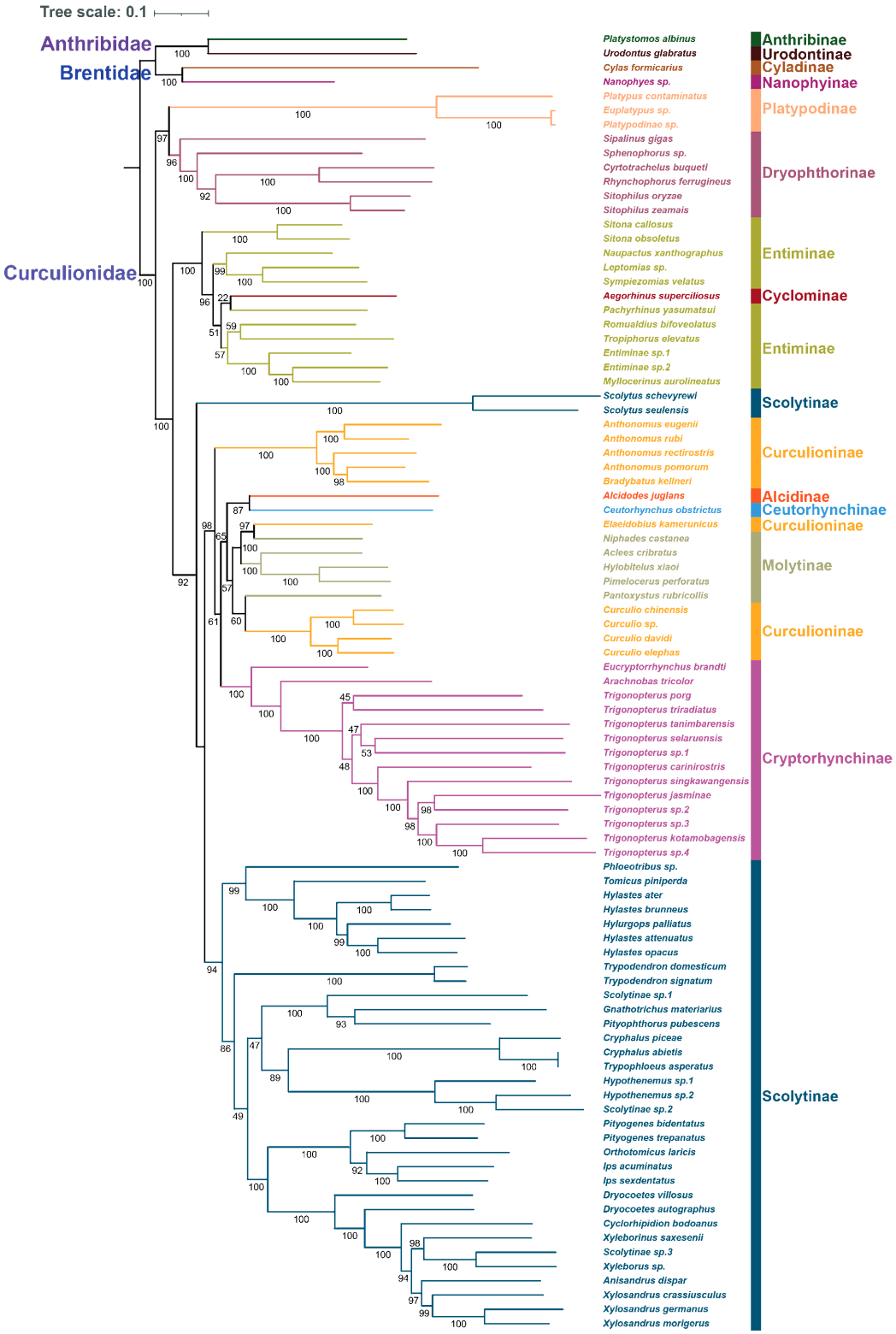

Figure 5

ML tree inferred using IQ-TREE and the PCG123 + rRNA dataset. Bootstrap support values (BS) are indicated on branches.

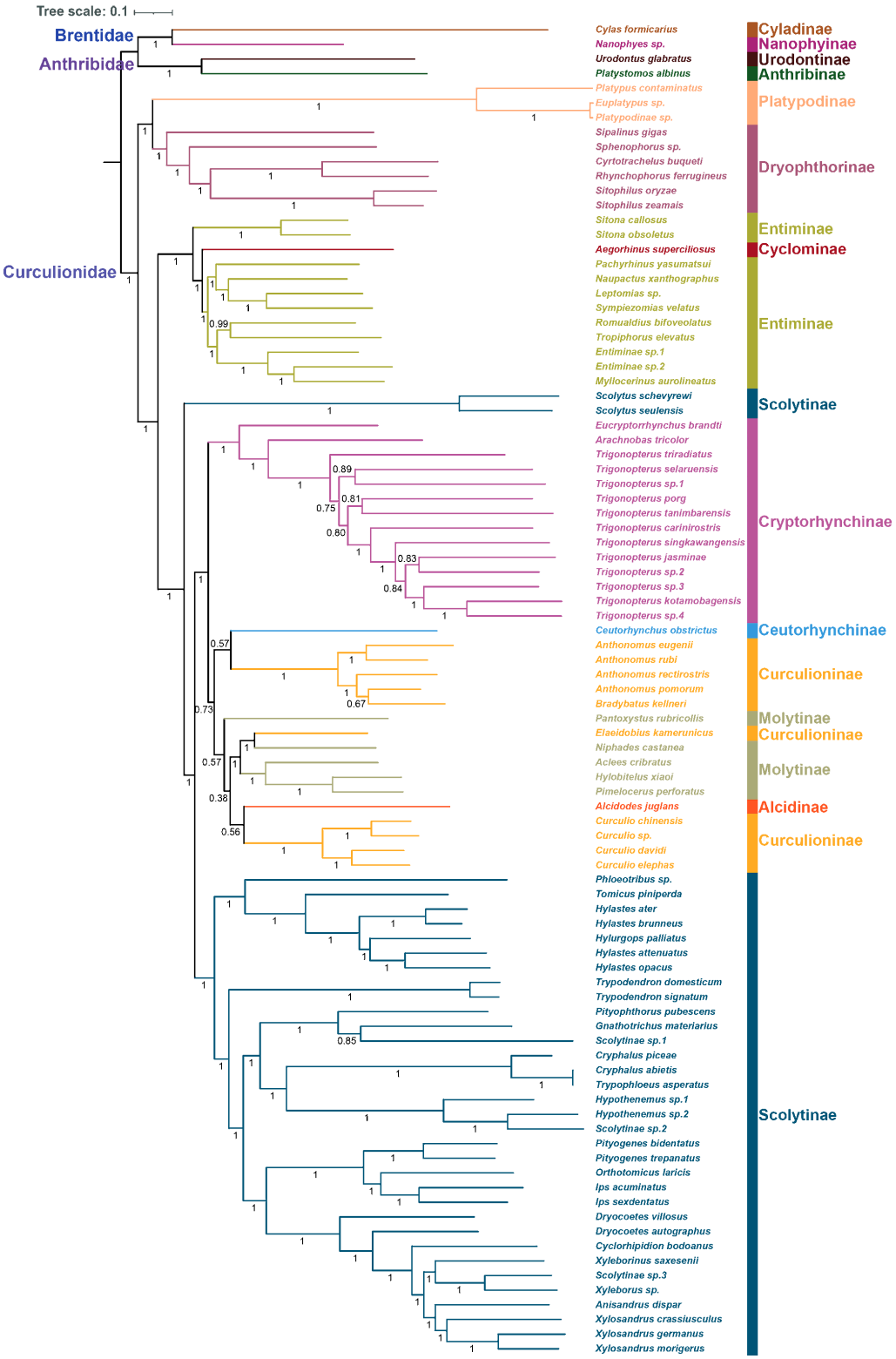

Figure 6

Bayesian tree inferred using MrBayes and the PCG123 + rRNA dataset. Bayesian posterior probabilities (PP) are indicated on branches.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- TableS2.docx
- TableS3.docx
- TableS4.docx
- TableS5.docx
- TableS6.docx
- TableS7.docx
- TableS8.docx
- TableS9.docx
- TableS10.docx
- TableS11.docx
- TableS12.docx