

Characterization of the Complete Mitochondrial Genome of *Epicauta impressicornis* (Coleoptera: Meloidae) and Its Phylogenetic Implications for the Infraorder Cucujiformia

Yang-Yang Liu, Zhi-Cheng Zhou, and Xiang-Sheng Chen¹

Institute of Entomology and Special Key Laboratory for Development and Utilization of Insect Resources of Guizhou, Guizhou University, Guiyang 550025, China and ¹Corresponding author, e-mail: chenxs3218@163.com

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Abstract

The complete mitochondrial genome (mitogenome) of *Epicauta impressicornis* Pic (Coleoptera: Meloidae) was determined. The circular genome is 15,713-bp long, and encodes 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and a control region (CR). The 13 PCGs start with the typical ATN codon and terminate with the typical stop codon TAA (*ND2*, *ND4L*, *ND6*, *ATP6*, *ATP8*, and *CYTb*), TAG (*ND1* and *ND3*), and T- (*COX1*, *COX2*, *COX3*, *ND4*, and *ND5*). The two rRNA genes (*rnr12S* and *rnr16S*) are encoded on the minority strand. All tRNAs genes except *trnS1* (*AGN*) are predicted to fold into the typical cloverleaf structure. The longest overlap (10 bp) is observed between *ATP8* and *ATP6*. CR mainly harbors a conserved poly-T stretch (15 bp), a short repeat unit (17 bp), some universal microsatellite-like repeats, and a canonical poly-A tail. Phylogenetic analysis using Bayesian inferences and maximum likelihood based on nucleotide and corresponding amino acid sequences of the 13 PCGs showed that *E. impressicornis* is closely related to *E. chinensis*, this relationship is supported within Cucujiformia belonging to Meloidae (Tenebrionoidea). Our results further confirmed the monophyly of Tenebrionoidea, Lymexyloidea, Curculionoidea, Chrysomeloidea, Cucujoidea, Coccinelloidea, and Cleroidea within Cucujiformia, and revealed the sister relationships of (Cleroidea + Coccinelloidea), (Lymexyloidea + Tenebrionoidea), and ((Chrysomeloidea + Cucujoidea) + Curculionoidea). We believe that the complete mitogenome of *E. impressicornis* will contribute to further studies on molecular bases for the classification and phylogeny of Meloidae or even Cucujiformia.

Key words: medicinal insect, mitogenome, meloidae, molecular phylogeny

As a traditional material in Chinese medicine, blister beetles have widely been used to treat various human diseases (e.g., edema, warts, molluscum, furuncles, hemorrhoids, and most types of cancers) because of the beetles' secretion of cantharidin which is an active toxic sesquiterpenoid (Carrel et al. 1993, Nikbakhtzadeh et al. 2007, National Pharmacopoeia Editorial Board 2010, Kim et al. 2013, Prasad and Verma 2013, Verma and Prasad 2013, Vakharia et al. 2018). Thus, blister beetles are of great medicinal value (National Pharmacopoeia Editorial Board 2010, Wang et al. 2010). The blister beetle *Epicauta impressicornis* Pic, which belongs to the family Meloidae, has attracted attention in research due to its importance in both pharmacology and agriculture (Pinto 1999, Bologna and Pinto 2002, Bologna et al. 2008, Wang et al. 2010, Du et al. 2016). Beetles of the genus *Epicauta* (one of many genera in the Meloidae family) show several interesting biological characteristics, such as hypermetamorphosis, adult clustering, and larval semi-parasitism (feeding on grasshopper eggs) with adult herbivory (feeding mainly on the flowers and leaves) (Pinto 1999; Bologna et al. 2008; Shintani

et al. 2011; Terao et al. 2012, 2015; Mu and Chen 2014, 2015; Shintani et al. 2017; Liu et al. 2018, 2019). In addition, the larvae and adults show beneficial and harmful effects in agriculture, respectively; larvae can be used for the biological control of grasshoppers, while adults are important pests of crops.

In recent years, the molecular phylogenies of several insect families have been studied in detail; however, there have been few studies on the molecular phylogeny of Meloidae species. Moreover, many studies of *E. impressicornis* have mainly focused on morphological taxonomy (Bologna and Pinto 2002, Bologna et al. 2008, Giulio et al. 2014, Pan and Bologna 2014, Liu et al. 2016, Liu et al. 2019), ecology and behavior (Shintani et al. 2011; Terao et al. 2012, 2015; Mu and Chen 2014, 2015; Shintani et al. 2017; Liu et al. 2018), molecular biology (Du et al. 2016, 2017; Jie et al. 2016; Wu et al. 2018a, b), and cantharidin characteristics (Jiang et al. 2019). Nevertheless, in the previous molecular phylogenetic studies of this family, Bologna et al. (2005, 2008) analyzed the phylogeny and evolution of Meloidae species in Europe using three gene fragments

[COI, 16S ribosomal RNA (rRNA), and ITS2] and Du et al. (2017) studied the phylogenetics of several Meloidae species based on comparative genomics.

In studies of molecular phylogeny of insects, mitochondrial genomes (mitogenomes) play an important role because of their rapid, comprehensive, and evolutionarily low levels of maternal inheritance and recombination (gene rearrangements), which are important for species identification, comparative genomics, population genetics, phylogenetic reconstruction, and evolutionary biology (Zhang and Hewitt 1997; Cameron 2014; Du et al. 2016, 2017). Mitochondrial DNA is a highly compact and a covalently closed circular double-stranded DNA molecule (~14–20 kb); it typically contains 37 genes, including 13 protein-coding genes (PCGs) [ATPase subunits 6 and 8 (*ATP6* and *ATP8*), cytochrome c oxidase subunits I–III (*COX1*–*COX3*), cytochrome B (*CYTB*), and NADH dehydrogenase subunits 1–6 and 4 I (*ND1*–6 and *ND4L*)] involved in oxidative phosphorylation and electron transport, two rRNAs, 22 transfer RNAs (tRNAs), and a noncoding control region (CR) of variable length comprising fundamental regulatory elements for transcription and replication (Wolstenholme 1992, Boore 1999). As of May 2019, only nine complete Meloidae mitogenomes had been published in GenBank (namely those of *E. aptera* Kaszab, *E. chinensis* Laporte, *E. gorhami* Marseul, *E. tibialis* Waterhouse, *Hycleus cichorii* (Linnaeus), *H. marcipoli* Pan & Bologna, *H. phaleratus* (Pallas), *Lytta caraganae* (Pallas), and *Mylabris aulica* (Menetries) [Coleoptera: Meloidae]) (Du et al. 2016, 2017; Jie et al. 2016; Wu et al. 2018b). An incomplete mitogenome of *H. chodscenticas* (Ballion) (Coleoptera: Meloidae), with many individual genes and incomplete gene fragments, has also been reported (Du et al. 2017). To date, the mitogenome of the pharmacologically and agriculturally important *E. impressicornis* has not been published. Currently, the general taxonomy of Chinese Meloidae is relatively disorganized due to simplistic species descriptions, lack of detail regarding morphological characteristics, and absence of molecular phylogenetic evidence from *Epicauta* species. Consequently, medicinal blister beetles are often misidentified in China, which seriously hinders the further development and use of these beetles as medicinal resources (Wang et al. 2010).

In the present study, we sequenced and annotated the complete mitogenome of *E. impressicornis*. Furthermore, we constructed phylogenetic trees based on the complete mitogenomes of 53 species of the beetle infraorder Cucujiformia, and five species of the infraorder Scarabaeiformia (as outgroups). The results of this study will contribute to understanding based on the characteristics of the *E. impressicornis* mitogenome, help determine the phylogenetic position of Meloidae, and reveal the phylogenetic relationships of members of Cucujiformia within Coleoptera, and facilitate future studies in all these areas.

Materials and Methods

Sample Collection, DNA Extraction, and Sequencing

Adults of *E. impressicornis* were collected from Luokun Village (106.58° E, 25.42° N), Luodian, Guizhou Province, China, in May 2018. All specimens were initially identified using morphological characteristics (Liu et al. 2016) and were immediately preserved in absolute ethanol and placed in a refrigerator at –20°C for 1 d in the Institute of Entomology of Guizhou University, before being transferred and stored in a refrigerator at –80°C in fresh absolute ethanol. Total genomic DNA was extracted from the samples using Takara Genome DNA Extraction Kits according to the manufacturer's instructions (Sangon Biotech, Shanghai, China), and DNA quality

was evaluated using 1% agarose gel electrophoresis. The complete mitogenomes were sequenced at OriGene (Beijing, China).

Sequence Assembly and Genome Annotation

Raw data were retrieved and qualified using FastQC (<http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/>), CLC Genomics Workbench 10.0 (CLC bio, Arhus, Denmark), Cutadapt (<http://cutadapt.readthedocs.org/>), and Pilon (Walker et al. 2014) using the default settings. Clean data were compared against reference mitochondrial genomes from GenBank (specifically *E. aptera*, *E. chinensis*, *E. gorhami*, and *E. tibialis*). Mitogenomes were reconstructed using Minimap2 (Li et al. 2018) and Nanopolish (<https://github.com/jts/nanopolish>) with the default parameters. Annotation of the mitochondrial genomes and comparative analyses were performed using GeSeq (Tillich et al. 2017) and Geneious R10 (Biomatters, Auckland, New Zealand), with BLAST against and related species from GenBank as the reference (Altschul et al. 1990, Kearse et al. 2012). Composition skewness was calculated according to the formulas AT-skew = $[A - T] / [A + T]$ and GC-skew = $[G - C] / [G + C]$ (Perna and Kocher 1995). Nucleotide composition and relative synonymous codon usage (RSCU) were analyzed and calculated using MEGA 7.0 (Kumar et al. 2016). Tandem repeats in the control region (CR) were predicted using the Tandem Repeats Finder program (<http://tandem.bu.edu/trf/trf.basic.submit.html>). The mitogenome sequence of *E. impressicornis* was deposited into GenBank under accession number MN082609. Transfer RNAs (tRNA) and secondary structures were identified and predicted using MITOS Web Server (Bernt et al. 2013). The tRNAs were visually inspected and compared with reference mitogenomes.

Phylogenetic Analysis

Phylogenetic analysis included sequences from the mitochondrial genomes of 58 species of Coleoptera, including 53 species of Cucujiformia, and five species of Scarabaeiformia, that were used as outgroups (Supp Table 1 [online only]). Nucleotide and amino acid sequences of 13 mitochondrial genes were aligned using Clustal X 1.83 (Thompson et al. 2017) with the default settings, and the resulting alignments were concatenated into two sets of sequences using SequenceMatrix 1.8 (Vaidya et al. 2011). The nucleotide matrix and amino acid matrix were used for phylogenetic analysis using two methods: Bayesian inference using MrBayes 3.2 (Ronquist et al. 2012) and maximum likelihood using IQ-TREE 1.6.2 (Nguyen et al. 2015). The best-fitting model (GTR + I + G) for the nucleotide sequences was selected using the Akaike information criterion (AIC) in jModeltest 2.1.10 (Darriba et al. 2012). MtArt + I + G + F was found to be the best-fitting model for the amino acid sequences using ProtTest 3.4 (Abascal et al. 2005). In the BI analysis, two runs of 10,000,000 generations were conducted for each matrix. Each set was sampled every 1,000 generations, with a burn-in of 25%. In the ML analysis, the node support values were assessed with 1,000 bootstrap resampling replicates. The resulting phylogenetic trees were visualized in FigTree 1.4.0 (Mousavi et al. 2014).

Results and Discussion

Mitogenome Organization and Base Composition

The complete mitogenome of *E. impressicornis* is 15,713 bp in length and is a typical closed circular DNA molecule. The length of this mitogenome lies between 15,645 bp of *E. aptera* (Jie et al. 2016) and the 15,717 bp of *E. chinensis* (Du et al. 2016). The *E. impressicornis* mitogenome is composed of 37 typical

mitochondrial genes including 13 PCGs, 22 tRNA genes, 2 rRNA genes, and a CR. This composition is similar to that observed in many insect mitogenomes: 23 genes (9 PCGs and 14 tRNAs) are encoded on the majority strand, and 4 PCGs, 8 tRNAs, and 2 rRNAs are encoded on the minority strand (Fig. 1, Table 1). The mitogenome gene order and orientation of *E. impressicornis* were the same those of other previously sequenced Meloidae and were also identical to ancestral arthropod mitogenomes (Bogdanowicz et al. 2000, Li et al. 2016). Specifically, the mitogenome contains 19 overlapped genes (49 bp), and the longest overlap (10 bp) is identical to that reported in other Meloidae species (Du et al. 2016, 2017), with the exception of *E. aptera* (Jie et al. 2016). Most of the gene overlaps appear in tRNA genes, which may be explained by the lower evolutionary constraints of these genes (Sheffield et al. 2008). Intergenic spacers in the *E. impressicornis* mitogenome have seven regions ranging from 1 to 17 bp (a total of 45 bp), with the longest region detected between *trnS2* (UCN) and *ND1* (Table 1). This particular intergenic spacer is similar to that previously reported in other Meloidae species (Du et al. 2016, 2017) and is common in to most Coleopteran mitogenomes (Sheffield et al. 2008, Yuan et al. 2016). Moreover, this intergenic spacer is apparently the basis for transcription termination site recognition using the transcriptional machinery (Sheffield

et al. 2008). The nucleotide composition of the *E. impressicornis* mitogenome is as follows: A = 36.90%, T = 34.00%, G = 11.30%, and C = 17.90%. The A+T and G+C contents are 70.90 and 29.10%, respectively. The AT-skew of the whole mitogenome is positive (0.04) and the GC-skew is negative (-0.23) (Supp Table 2 [online only]); this finding indicates a strand compositional bias characterized by a strong excess of C over G nucleotides and a slight excess of A over T nucleotides, which also corresponds well to the A + T bias generally observed in Meloidae mitogenomes (Du et al. 2017).

Protein-Coding Genes

In the mitogenome of *E. impressicornis*, the 13 typical PCGs (11,115 bp in length) commonly found in other reported Meloidea species (Du et al. 2016, 2017) and previously described in the Introduction were found, i.e., the seven NADH genes, two ATPase genes, and four cytochrome dehydrogenase genes (Fig. 1, Supp Table 2 [online only]), all of which are commonly found in other reported Meloidea species (Du et al. 2016, 2017). Nine of the 13 PCGs are encoded on the majority strand (*COX1*, *COX2*, *COX3*, *CYTB*, *ND2*, *ND3*, *ND6*, *ATP6*, and *ATP8*) and are on the minority strand (*ND1*, *ND4*, *ND4L*, and *ND5*) (Fig. 1, Table 1). All 13 PCGs start with a typical ATN start codon. The conventional termination codons

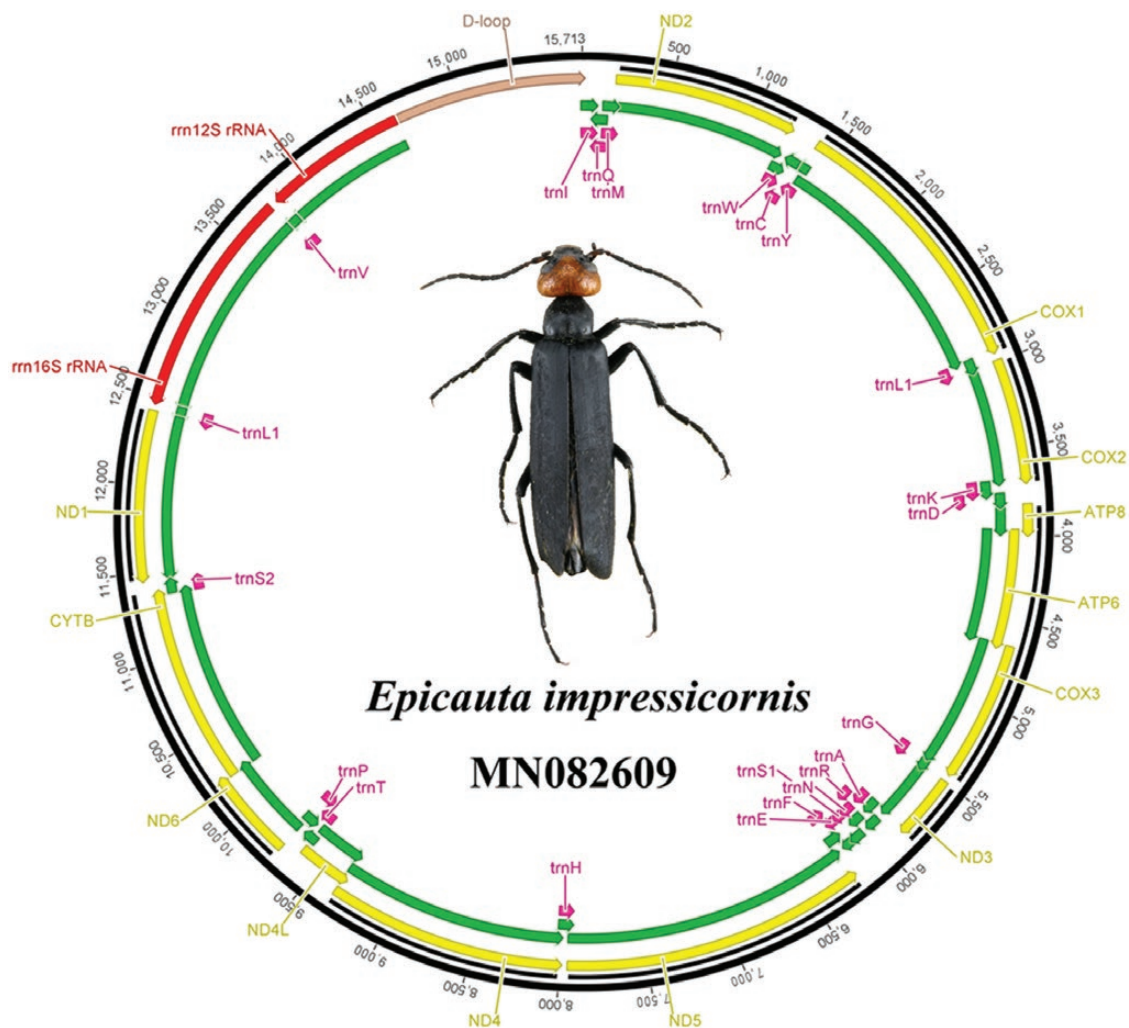


Fig. 1. Gene map of the mitogenome of *E. impressicornis*. Gene abbreviations are as follows: *COX1–3*, cytochrome c oxidase subunits 1–3; *ATP6* and *ATP8*, ATP synthase subunits 6 and 8; *CYTB*, cytochrome B; *ND1–6* and *ND4L*, NADH dehydrogenase subunits 1–6 and 4 L. In addition, *trnS1*, *trnS2*, *trnL1*, and *trnL2* denote codons *tRNA-Ser* (AGN), *tRNA-Ser* (UCN), *tRNA-Leu* (CUN), and *tRNA-Leu* (UUR), respectively.

Table 1. Summary of the mitogenome of *E. impressicornis*

Gene	Strand	Location	Size (bp)	Anticodon	Start codon	Stop codon	Intergenic nucleotides
<i>trnI</i>	+	1–66	66	GAT			
<i>trnQ</i>	–	63–132	70	TTG			4(–)
<i>trnM</i>	+	132–200	69	CAT			1(–)
<i>ND2</i>	+	201–1214	1,014		ATA	TAA	
<i>trnW</i>	+	1,213–1,280	68	TCA			2(–)
<i>trnC</i>	–	1,280–1,343	64	GCA			1(–)
<i>trnY</i>	–	1,347–1,410	64	GTA			3(+)
<i>COX1</i>	+	1,403–2,945	1,543		ATT	T	8(–)
<i>trnL2(UUR)</i>	+	2,946–3,010	65	TAA			
<i>COX2</i>	+	3,011–3,698	688		ATA	T	
<i>trnK</i>	+	3,698–3,770	73	CTT			1(–)
<i>trnD</i>	+	3,769–3,833	65	GTC			2(–)
<i>ATP8</i>	+	3,843–3,995	153		ATA	TAA	9
<i>ATP6</i>	+	3,986–4,657	672		ATG	TAA	10(–)
<i>COX3</i>	+	4,657–5,437	781		ATG	T	1(–)
<i>trnG</i>	+	5,438–5,501	64	TCC			
<i>ND3</i>	+	5,502–5,855	354		ATT	TAG	
<i>trnA</i>	+	5,854–5,918	65	TGC			2(–)
<i>trnR</i>	+	5,918–5,981	64	TCG			1(–)
<i>trnN</i>	+	5,981–6,045	65	GTT			1(–)
<i>trnS1(AGN)</i>	+	6,046–6,102	57	TCT			
<i>trnE</i>	+	6,103–6,164	62	TTC			
<i>trnF</i>	–	6,163–6,226	64	GAA			2(–)
<i>ND5</i>	–	6,227–7,937	1,711		ATT	T	
<i>trnH</i>	–	7,937–8,002	66	GTG			1(–)
<i>ND4</i>	–	8,002–9,334	1,333		ATG	T	1(–)
<i>ND4L</i>	–	9,328–9,615	288		ATG	TAA	7(–)
<i>trnT</i>	+	9,618–9,680	63	TGT			2(+)
<i>trnP</i>	–	9,680–9,745	66	TGG			1(–)
<i>ND6</i>	+	9,747–10,238	492		ATT	TAA	1(+)
<i>CYTB</i>	+	10,238–11,377	1,140		ATG	TAA	1(–)
<i>trnS2(UCN)</i>	+	11,376–11,443	68	TGA			2(–)
<i>ND1</i>	–	11,461–12,411	951		ATT	TAG	17(+)
<i>trnL1(CUN)</i>	–	12,412–12,475	64	TAG			
<i>rrn16S</i>	–	12,482–13,747	1,266				6(+)
<i>trnV</i>	–	13,755–13,823	69	TAC			7(+)
<i>rrn12S</i>	–	13,824–14,613	790				
Control region (CR)		14,614–15,713	1,100				

In the column for intergenic length, a positive sign indicates the interval in base pairs between genes, while the negative sign indicates overlapping base pairs between genes.

TAA and TAG are assigned to six PCGs (*ND2*, *ND4L*, *ND6*, *ATP6*, *ATP8*, and *CYTB*) and two PCGs (*ND1* and *ND3*), respectively, whereas *COX1*, *COX2*, *COX3*, *ND4*, and *ND5* genes show T- as the incomplete stop codon (Table 1). Similar situations have been observed in many other insect species (Boore et al. 2001, Kim et al. 2006, Du et al. 2016), incomplete stop codons may emerge as functional stop codons via polyadenylation processes and polycistronic transcription cleavage; moreover, this may function to minimize gene overlaps and intergenic spacers (Ojala et al. 1981).

The results of RSCU analysis for the 13 PCGs, comprising 3,705 codons excluding start and stop codons, in the *E. impressicornis* mitogenome are presented in Fig. 2 and Supp Table 3 [online only]. In addition, codon distribution analysis (Fig. 3, Supp Table 3 [online only]) demonstrated that UUU (222 incidences), UUA (268), AUA (268), and AUA (206) were the most frequently used codon families (they occurred >200 times and accounted for 26.02% of the codons), followed by AAU(127) and UAU (119). All these codons consist of A or T nucleotides, which is indicative of the biased usage of A and T nucleotides in *E. impressicornis* PCGs. Many other Coleopteran

species have a similar pattern of codon usage in their mitogenomes (Kim et al. 2009, Du et al. 2016, Wang et al. 2019). Nine codons in the 13 PCGs (UCG, ACG, GCG, UAA*, UAG*, UGC, CGC, CGG, and AGG) do not exceed 10 instances. Furthermore, the AT and GC-skews, of these PCGs were negative (Supp Tables 2 and 3 [online only]).

Transfer and Ribosomal RNAs

The typical set of 22 tRNA genes identified within the *E. impressicornis* mitogenome, which showed a total length of 1,441 bp, varied from 57 bp (*trnS1*) to 73 bp (*trnK*) in length (Table 1 and Supp Table 2 [online only]). The positions of tRNAs in the mitogenome are usually conserved across insect species (Du et al. 2016, 2017; Wang et al. 2019). In *E. impressicornis*, these genes had an A+T content of 72.90% and a positive AT (0.04) and GC (0.18) skews (Supp Table 2 [online only]). Fourteen tRNA genes are encoded on the majority strand and eight on the minority strand. Anticodons of all of the tRNA genes were identical to those found in other Meloidae mitogenomes (Du et al. 2016, 2017; Jie et al.

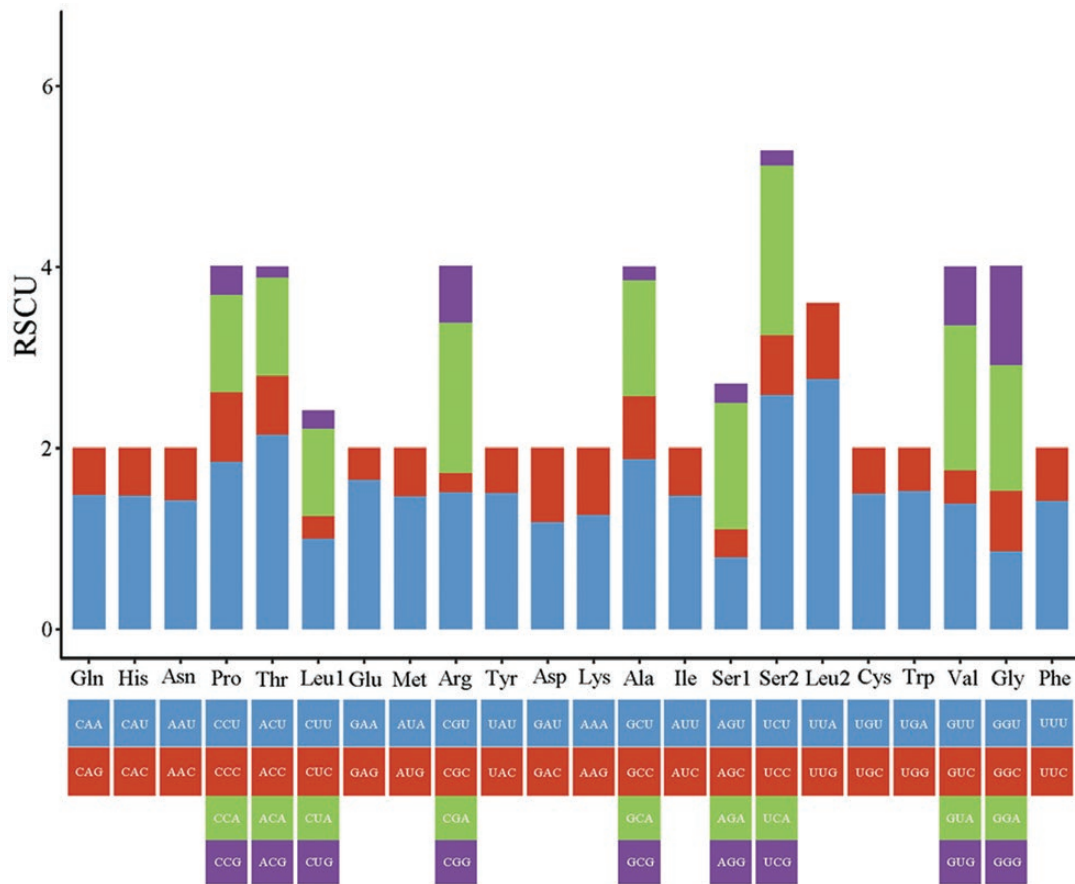


Fig. 2. Relative synonymous codon usage (RSCU) in the mitogenome of *E. impressicornis*.

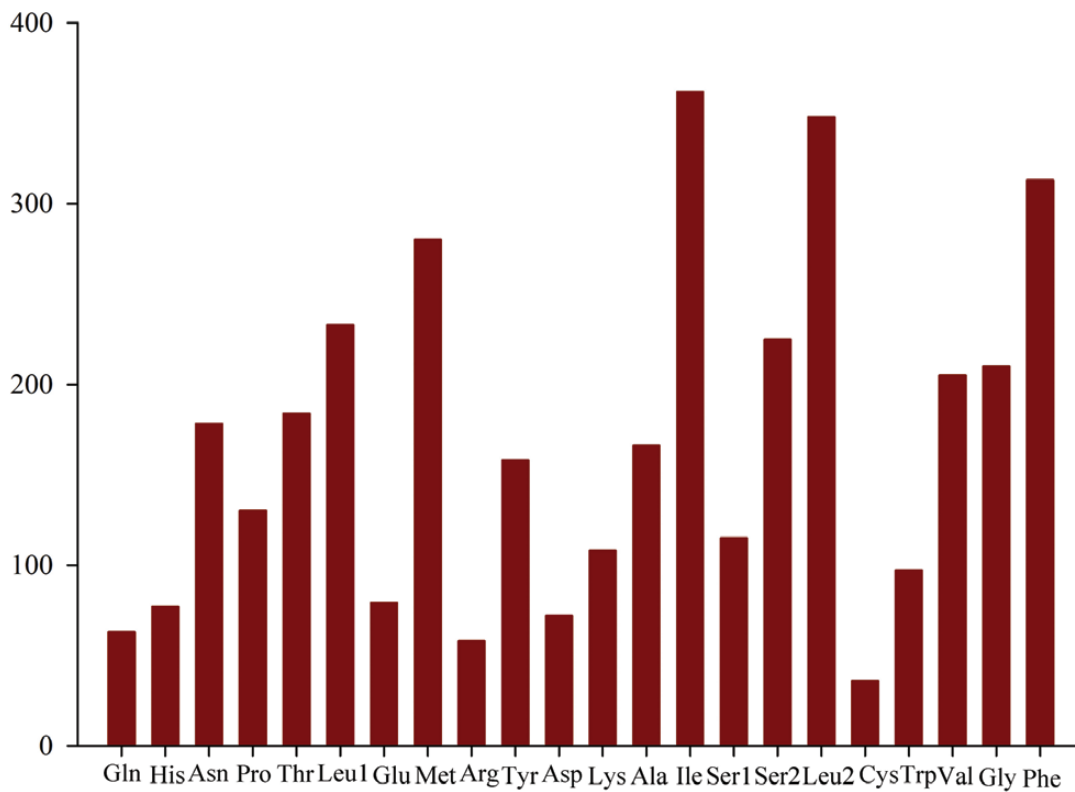


Fig. 3. Amino acid composition of the mitogenome of *E. impressicornis*.

2016). Most of the identified tRNA genes had a typical cloverleaf secondary structure, with the exception of *trnS1* (AGN), which had lost the dihydrouridine (DHU) arm (Supp Fig. 1 [online only]). Similarly, in many non-Meloidae insect mitogenomes, *trnSer* (AGN) lacks the DHU arm and cannot fold into the emblematic cloverleaf structure; thus, this is a common phenomenon (Wolstenholme 1992; Cameron 2014; Du et al. 2016, 2017; Jie et al. 2016; Wang et al. 2019). However, increasing variation is usually accompanied more compensatory base changes in stems, which results in tRNAs that are more or less unconserved (Du et al. 2017).

Two rRNA genes were discovered on the minority strand (*rrn12S* and *rrn16S*) of the *E. impressicornis* mitogenome; these genes are typically observed in other Meloidae species and in many other insect mitogenomes (Du et al. 2016, 2017; Wang et al. 2019). In total, these rRNA genes were 2,056 bp in length: *rrn16S* (1,266 bp in length) was found to be situated between *trnL1* (CUN) and *trnV*, whereas *rrn12S* (790 bp in length) was located between *trnV* and the CR (Table 1). The gene *rrn16S* was assumed to supplement the blanks between *trnL1* (CUN) and *trnV* (Fig. 1). The terminal region of the rRNA genes may have extended to the boundaries of flanking genes because it was difficult to accurately resolve by DNA sequence alone (Boore 2001, 2006). The boundary between *rrn12S* and the putative CR can be determined by an alignment with homologous sequences in other Meloidae insects

(Boore and Brown 2000; Jie et al. 2016; Du et al. 2016, 2017). For rRNAs in *E. impressicornis*, the AT-skew was negative (−0.06) and the GC-skew was positive (0.35), with a high A+T content in the mitogenome (74.60%) (Supp Table 2 [online only]). The locations of *rrn12S* and *rrn16S* in the *E. impressicornis* mitogenome each correspond to those in the mitogenomes of other known Meloidae species (Supp Table 4 [online only]); this finding demonstrates that rRNAs are conserved in Meloidae and possess sites that might have played an important role in phylogenetic evolution (Du et al. 2016, 2017; Jie et al. 2016).

Control Region

The mitochondrial CR is involved in the initiation and regulation of replication and transcription in insects (Zhang et al. 1995, Zhang and Hewitt 1997, Fernandez-Silva et al. 2003). The length of the CR in the *E. impressicornis* mitogenome, which was 1,100 bp, lies between the lengths of *L. caraganae* (1,015 bp) and *M. aulica* (1,139 bp) (Supp Table 4 [online only]). It was found to be located between *rrn12S* and *trnI*. With 884 bp, it exhibited the highest A+T content (80.36%) of the entire of *E. impressicornis* mitogenome, which is in agreement with other Meloidae mitogenomes. The overall AT-skew and the GC-skew in the CR were 0.04 and −0.18, respectively (Fig. 1, Supp Table 2 [online only]). Since high A+T content limits the utility of genes as molecular markers, it is possible that the PCGs

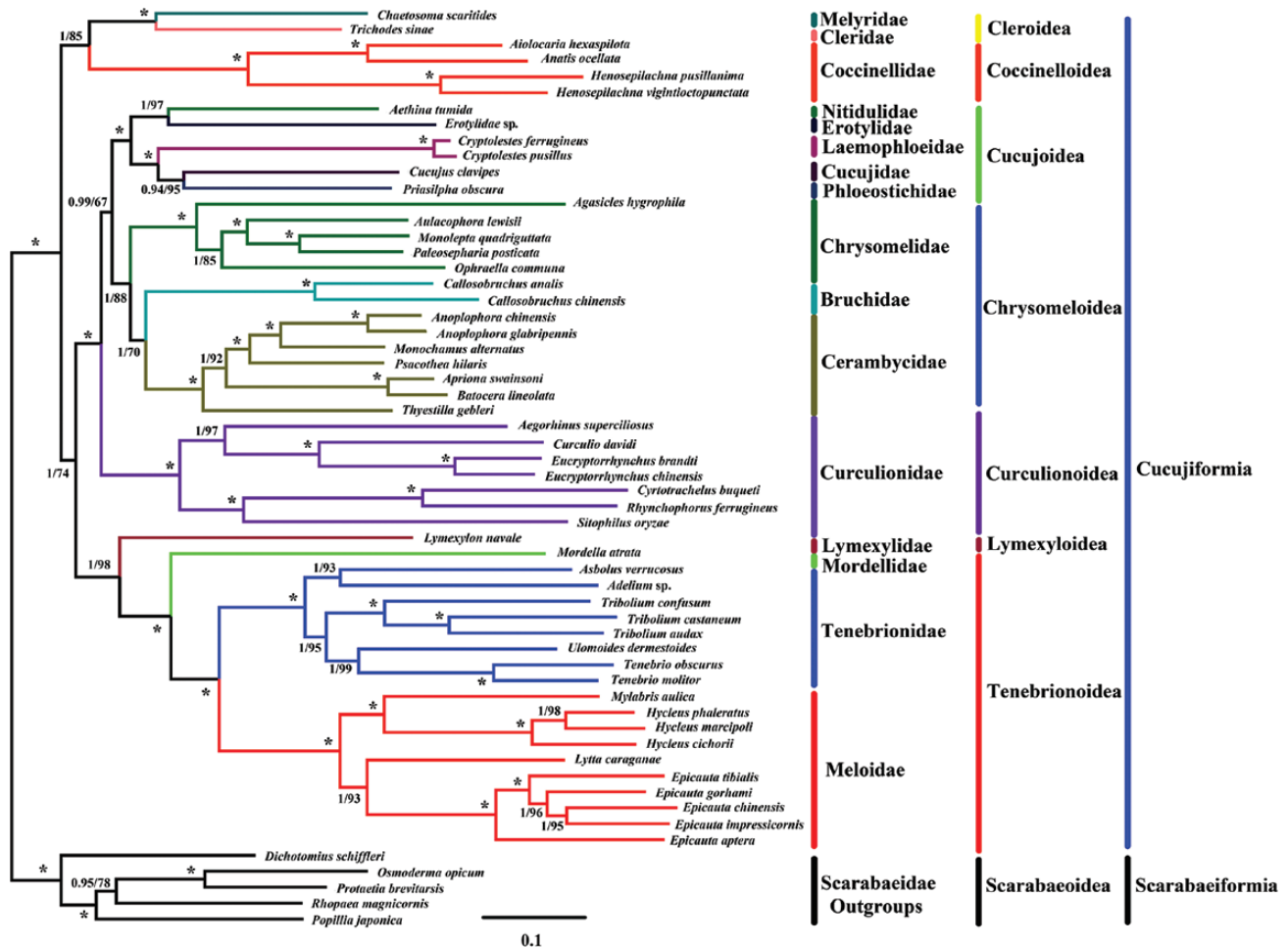


Fig. 4. Phylogeny of *E. impressicornis*. The phylogenetic tree was inferred from the nucleotide sequences of 13 PCGs in the mitogenome using Bayesian Inferences (BI) and Maximum Likelihood (ML) methods. Numbers on branches indicate BI posterior probability (PP, left) and ML bootstrap support (BS, right). An asterisk indicates PP = 1.0 and BS = 100.

might be more variable than the CR (Zhang and Hewitt 1997, Du et al. 2016). A conserved poly-T stretch (15 bp) was found in the CR of the *E. impressicornis* mitogenome, and it was comparable to that in other Meloidae mitogenomes (Du et al. 2016, Jie et al. 2016); this has been reported to function as a possible recognition site for the origin of the DNA replication minor strand (Zhang and Hewitt 1997, Andrews et al. 1999, Saito et al. 2005, Kim et al. 2009). A tandem repeat sequence was identified centrally in the *E. impressicornis* CR with the consensus pattern ATTAATAATGATTATAT (17 bp), which was repeated by base insertions/deletions/mismatches. Given that other published Meloidae mitogenomes do not have tandem repeats, this feature might provide a useful molecular marker for *E. impressicornis* in relation to Meloidae species identification (except in phylogeographic and population genetics studies) (Zhang and Hewitt 1997; Du et al. 2016, 2017). Microsatellite-like elements such as (AT)₃ and (AT)₈ also appeared in the CR between the tandem repeat sequence and trnI. In addition, as in other Meloidae mitogenomes (Du et al. 2016), poly-A was identified at the end of the CR (Supp Fig. 2 [online only]).

Phylogenetic Analysis

In the present study, phylogenetic analyses were based on the nucleotide and amino acid sequences of the 13 PCGs from the complete mitogenomes of 53 species of Cucujiformia (Figs. 4 and 5). Previous phylogenetic studies have shown that different

datasets and inference methods affect the topological structure of Cucujiformia (Beutel and Haas 2000, Timmermans et al. 2015, Yuan et al. 2016). Thus, similarities and differences exist in different phylogenetic trees.

Here, BI trees provided significantly higher support values than ML trees in the same dataset, especially at branches that involved the superfamily-level relationships of the 13 PCG nucleotide sequences at the support values (PP/BS = 0.99/67) of (Chrysomeloidea + Cucujoidea) (Fig. 4) and the 13 PCGs amino acid sequences at the support values (PP/BS = 1/57) of (Coccinelloidea + Cleroidea) + (Lymexyloidea + Tenebrionoidea) (Fig. 5). However, the lack of resolution at some nodes affected the tree topology; these results might have been due to limited taxon sampling, which is one of the most important determinants of accurate phylogenetic inference, particularly in species-rich lineages (Zwick and Hillis 2002, Rosenberg 2007).

Unsurprisingly, *E. impressicornis* clustered with the genus *Epicauta* in Meloidae. In particular, *E. impressicornis* had a close relationship with *E. chinensis*. Similar to previous findings (Du et al. 2017), phylogenetic analyses among representative members of Meloidae resulted in two major clades: *Epicauta* and *Lytta* formed one clade (a sister group) while *Hycleu* and *Mylabris* formed the other. At the family level, Meloidae, Tenebrionidae, Mordellidae, and Lymexylidae formed one clade; Cleridae and Melyridae formed a sister group; while Cerambycidae, Chrysomelidae, and Bruchidae formed the final clade. These results were congruent with previous

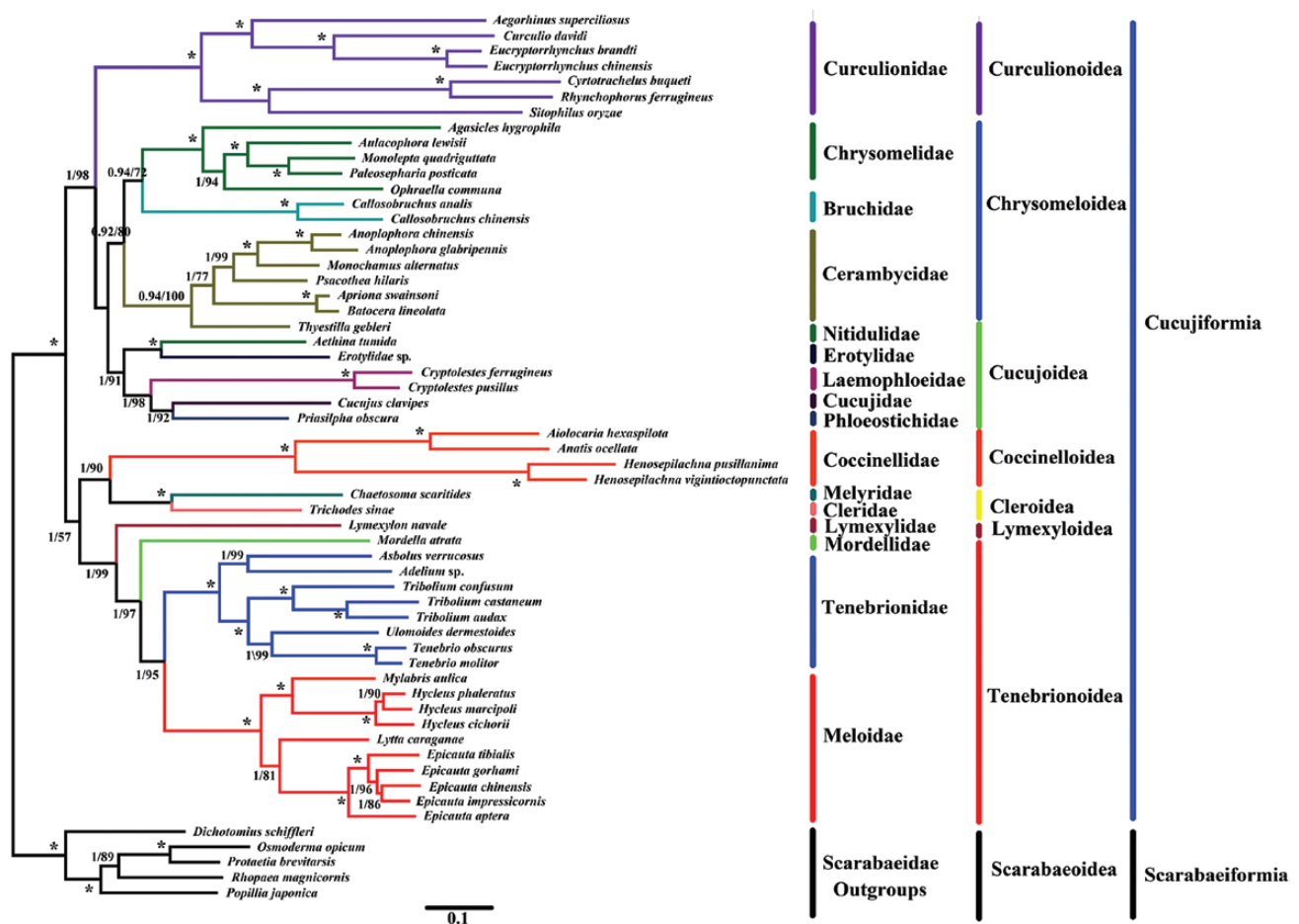


Fig. 5. Phylogeny of *E. impressicornis*. The phylogenetic tree was inferred from the amino acid sequences of the 13 PCGs in mitogenome using Bayesian Inferences (BI) and Maximum Likelihood (ML) methods. Numbers on branches indicate BI posterior probability (PP, left) and ML bootstrap support (BS, right). An asterisk indicates PP = 1.0 and BS = 100.

phylogenetic studies using mitogenomes (Timmermans et al. 2015, Yuan et al. 2016).

At the superfamily level, the phylogenetic relationships in Cucujiformia were somewhat ambiguous. The phylogenetic topologies of Coccinelloidea, Cleroidea, Curculionoidea, Chrysomeloidea, Cucujoidea, Lymexyloidea, and Tenebrionoidea were well-established and formed their respective clades. Three sister groups were identified, (Cleroidea + Coccinelloidea), (Lymexyloidea + Tenebrionoidea), and ((Chrysomeloidea + Cucujoidea) + Curculionoidea), which were consistent with previous phylogenetic research using the mitogenomes (Timmermans et al. 2010, Gunter et al. 2014, Timmermans et al. 2015, Yuan et al. 2016). However, the position of (Coccinelloidea + Cleroidea) was not stable. For example, in the nucleotide sequence analysis, (Coccinelloidea + Cleroidea) and (Lymexyloidea + Tenebrionoidea) collectively constituted a clade (Fig. 4). However, in amino acid sequence analysis (Coccinelloidea + Cleroidea) and ((Lymexyloidea + Tenebrionoidea) + ((Chrysomeloidea + Cucujoidea) + Curculionoidea)) comprised a clade (Fig. 5). Thus, the phylogenetic relationships in Cucujiformia were distinctive in the nucleotide sequence analysis (Fig. 4) and the amino acid sequence analysis (Fig. 5). Although this finding was inconsistent with previous mitogenome-based phylogenetic research (Timmermans et al. 2015, Yuan et al. 2016), (Lymexyloidea + Tenebrionoidea) and ((Cucujoidea + Chrysomeloidea) + Curculionoidea) could be well clustered into a sole branch. As previously reported in mitogenomic studies, there are some well-supported populations and some inconsistent relationships among major lineages in Cucujiformia. Cucujiformia is a diverse clade and contains the highest abundance of polyphaga species in Coleoptera (Sheffield et al. 2009, Bocak et al. 2014, Timmermans et al. 2015, Du et al. 2016, Li et al. 2016). Consequently, it remains a challenge to construct a well-supported Cucujiformia system.

At present, a successful analysis of the phylogenetic relationships in Cucujiformia based on either morphological or molecular characteristic does not exist. Indeed, many phylogenetic studies of Coleoptera have failed to provide strong support or to identify and infer phylogenetic relationships within Cucujiformia (Timmermans et al. 2015, Yuan et al. 2016). The phylogenetic relationships reported in the present study differed from those reported in previous phylogenetic studies; this might be due to differences in datasets (Marvaldi et al. 2008, Kim et al. 2009, Coates 2014) and/or the inconsistency among different mitochondrial regions detected in diverse species (Meiklejohn et al. 2014). Nevertheless, our study will be useful for providing a molecular basis for the classification and phylogeny of Cucujiformia. Considering the diversity of Cucujiformia and the limitations of the current molecular information, the accurate phylogeny within Cucujiformia will require more molecular markers (either from mitochondrial data or nuclear segments) and additional samples.

Supplementary Data

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

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