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Structural Features and Phylogenetic Implications of Three New Mitochondrial Genomes of Blister Beetles (Coleoptera: Meloidae)

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Abstract

The mitochondrial genome sequences of *Denierella emmerichi*, *Epicauta curvispina*, and *Meloe poggii* were determined. Their mitochondrial genomes were found to contain 37 genes (13 protein-coding genes [PCGs], 22 tRNA genes, and 2 rRNAs), of which 4 PCGs, 8 tRNA genes, and 2 rRNAs are encoded by the N-strand, and the remaining genes are encoded by the J-strand. The mitochondrial genomes of *D. emmerichi*, *E. curvispina*, and *M. poggii* are 15,702 bp, 15,813 bp, and 15,626 bp in length, respectively, and their guanine–cytosine contents are 28%, 33%, and 36%, respectively. The 13 PCGs of *D. emmerichi*, *E. curvispina*, and *M. poggii* use ATN as the standard start codon and TAA, TAG, and T as the stop codons. The Bayesian inference and maximum likelihood phylogenetic analysis results based on the 13 PCGs and 13 PCGs + 2rRNAs datasets of the mitochondrial genomes of the Meloidae support *Epicauta* (Coleoptera: Meloidae) ([*D. emmerichi*, *E. curvispina*, *E. ruficeps*, *E. aptera*] + [*E. chinensis*, *E. impressicornis*, *E. gorhami*, *E. tibialis*]). We believe that this research enriches the literature on the mitochondrial genomics of Meloidae and serves as a foundation for the further study of the phylogenetic relationships and characterization of Meloidae and Coleoptera.

Key words: medicinal insect, Meloidae, mitochondrial genome, phylogenetics, genomics

Meloidae (Coleoptera: Tenebrionoidea) is a medium-sized family that contains approximately 125 genera comprising more than 3,000 species (Du et al. 2016; Bologna et al. 2008). Blister beetles, which belong to this family, secrete cantharidin, which is a sesquiterpene substance with important medicinal value that has been used in traditional Chinese medicine (Liu et al. 2018, 2020). Studies on blister beetles have primarily focused on their morphological taxonomy (Bologna et al. 2008; Bologna and Pinto 2002; Yang and Ren 2007; Batelka and Hájek 2015; Pan and Ren 2018; Li et al. 2020) and ecology (Liu et al. 2018; Shintani et al. 2010; Terao et al. 2012; Terao et al. 2015; Okano et al. 2015; Shintani et al. 2017). However, some blister beetles have very similar morphologies, and it is difficult to distinguish them using traditional methods.

In recent years, mitochondrial genomics has been applied to study blister beetles (Du et al. 2016, 2017; Liu et al. 2020). The animal mitochondrial genome has become an important molecular marker in population and evolutionary biology because of many features that can be used to distinguish similarities or dissimilarities among species, including stable gene composition, relatively conservative gene arrangement, general maternal inheritance, and, rarely, recombination (Wolstenholme 1992). The mitochondrial genome has been widely used in studies of mitochondrial genome evolution, phylogenetic inference, species origin identification, gene drift, biogeography, population structure, dynamic analysis, molecular evolution, etc. (Avise 1994; Zhang and Hewitt 1997; Du et al. 2016; Liu et al. 2020). The mitochondrial genome of insects is a double-stranded closed-loop molecule containing 37 genes (13 protein-coding genes [PCGs], 22 transfer RNA [tRNA] genes, and 2 ribosomal RNA [rRNA] genes, [rrnS and rrnL]) and a noncoding control region (Boore 1999). Therefore, mitochondrial genomics can be employed to explore the phylogenetic relationships between some populations of Meloidae and provide reference materials for the taxonomic study of blister beetles.

In this study, we sequenced, assembled, and annotated the mitochondrial genomes of three blister beetles and analyzed the basic structural characteristics of their genomes. Our findings supplement the literature on the mitochondrial genomics of Meloidae, contribute to the classification and identification of blister beetles, and are of great value in the study of the genetic and evolutionary biology of Meloidae.

Materials and Methods

Species Collection and Taxonomic Identification

Adult specimens of *D. emmerichi* and *E. curvispina* were collected from Taoyuan County (110.51°E, 28.24°N), Hunan Province,
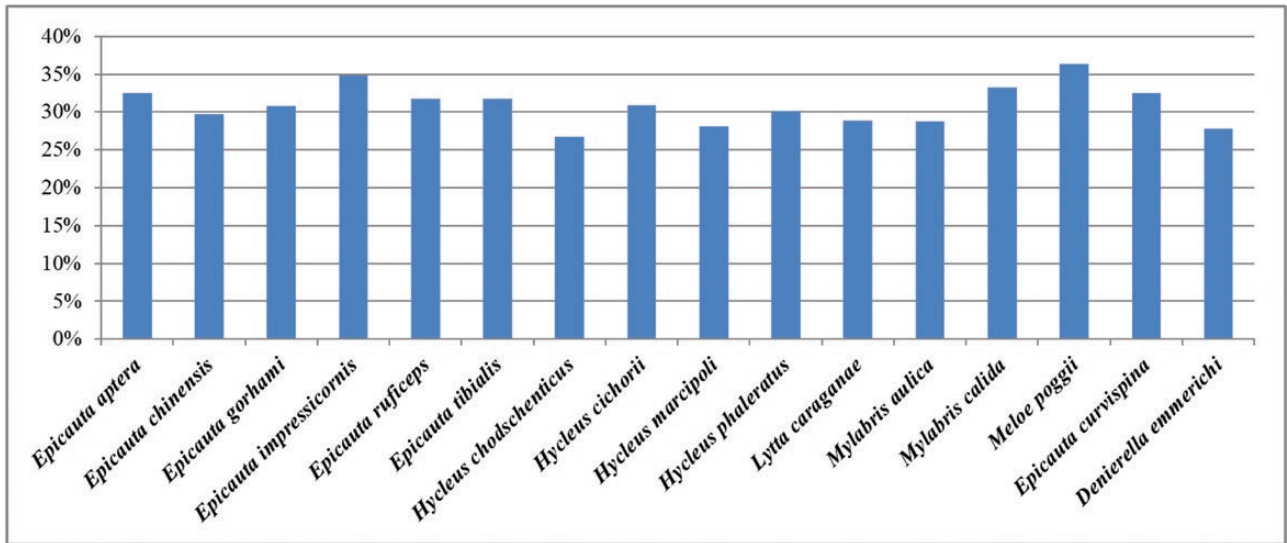
Fig. 1. Guanine–cytosine contents of the mitochondrial genomes of different species.
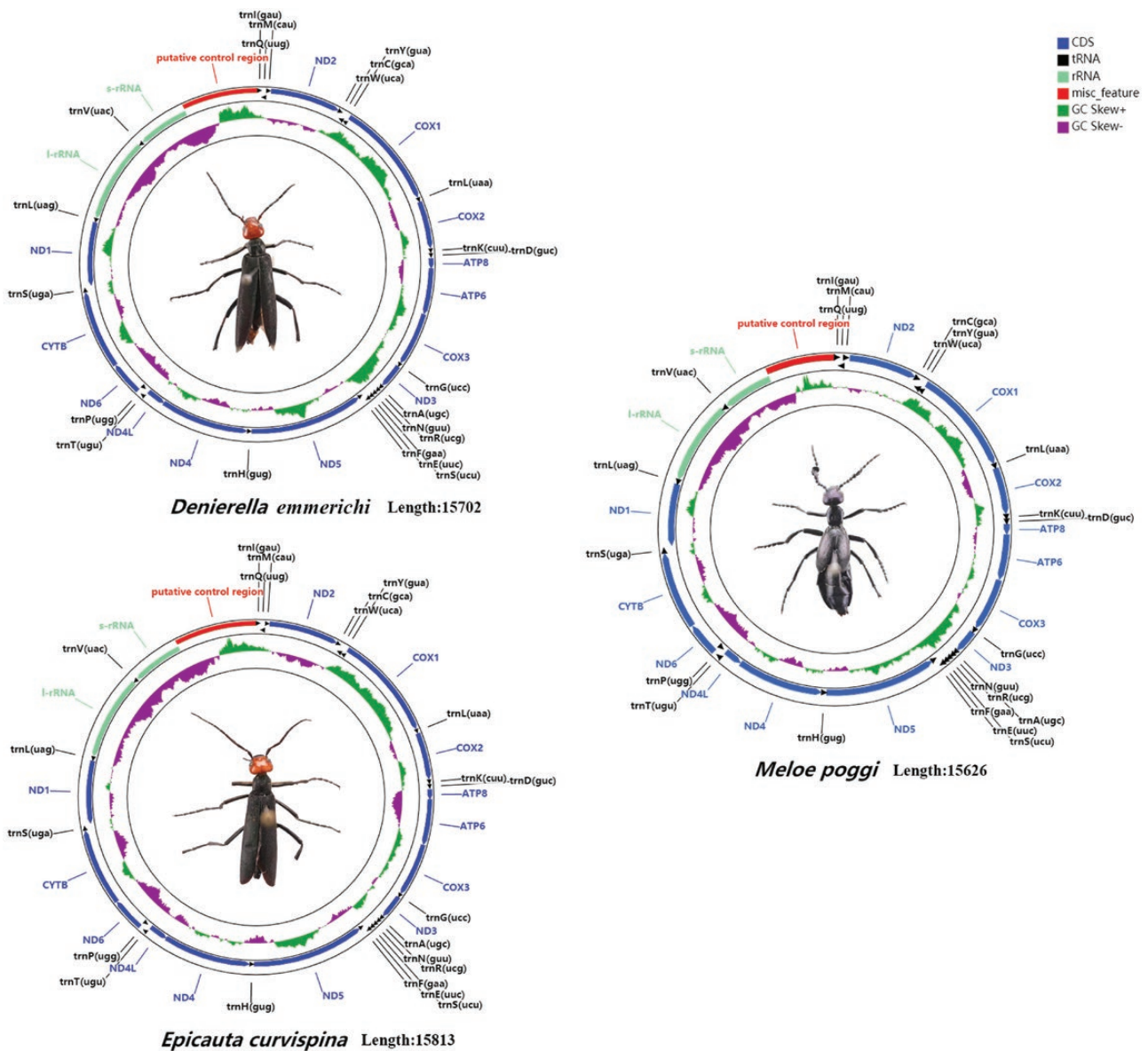

Fig. 2. Gene map of the mitochondrial genomes of *D. emmerichi*, *E. curvispina*, and *M. poggii*. Gene abbreviations are as follows: COX1–3, cytochrome c oxidase subunits 1–3; ATP6 and ATP8, ATP synthase subunits 6 and 8; CYTB, cytochrome B; ND1–6 and ND4L, NADH dehydrogenase subunits 1–6 and 4 L.

China, in June 2020; adult specimens of *M. poggii* were collected from Dêgê County (99.29°E, 31.88°N), Sichuan Province, China, in September 2020. The specimens were soaked in anhydrous ethanol and stored at –80°C at the Institute of Entomology, Guizhou University, Guiyang, China. The specimens were identified based on morphological analysis (Yang and Ren 2007; Liu et al. 2016; Pan and Bologna 2021).

DNA Extraction and Sequencing

The abdomen of the specimens was removed; DNA was extracted using a Blood Genome DNA Extraction Kit (TaKaRa, Japan), and 1% agarose gel electrophoresis was performed. The integrity and purity of the DNA samples were determined using a Nanodrop spectrophotometer. Qualified DNA samples were sent to Berry Genomics (China) for sequencing. A single sample was independently built, and resequencing was performed using the Illumina NovaSeq 6000 sequencing platform with a 300-bp insert, sequencing mode set to 150 PE, data volume output of each sample ≥6 Gb, and average data quality of Q30 ≥85%.

Genome Assembly, Annotation, and Analysis

The assembly, preliminary annotation, and visualization of the mitochondrial genome were completed using MitoZ version 2.4 (Meng et al. 2019). The relative codon usage (RSCU) frequency and nucleotide composition of the mitochondrial genome were analyzed using PhyloSuite version 1.2.2 (Zhang et al. 2020). The graph of the RSCU frequency was constructed using the fmsb package in R 3.6.1. The locations and secondary structures of tRNA genes were predicted using the tRNAscan-SE search server (http://lowelab.ucsc.edu/tRNAscan-SE) and the MITOS web server (http://mitos.bioinf.uni-leipzig.de/index.py), with an invertebrate codon predictor (Lowe and Eddy 1997). The heat map of the guanine–cytosine (GC) content of different gene fragments among different species was constructed using TBtools (Chen et al. 2020).

Phylogenetic Analysis

Based on the complete mitochondrial genome sequence, the combined use of MEGA version 7.0.26 (Kumar et al. 2016) and SequenceMatrix version 1.7 (Vaidya et al. 2011) was employed to obtain the nucleotide sequences of the datasets 13 PCGs and 13 PCGs + 2 rRNAs.
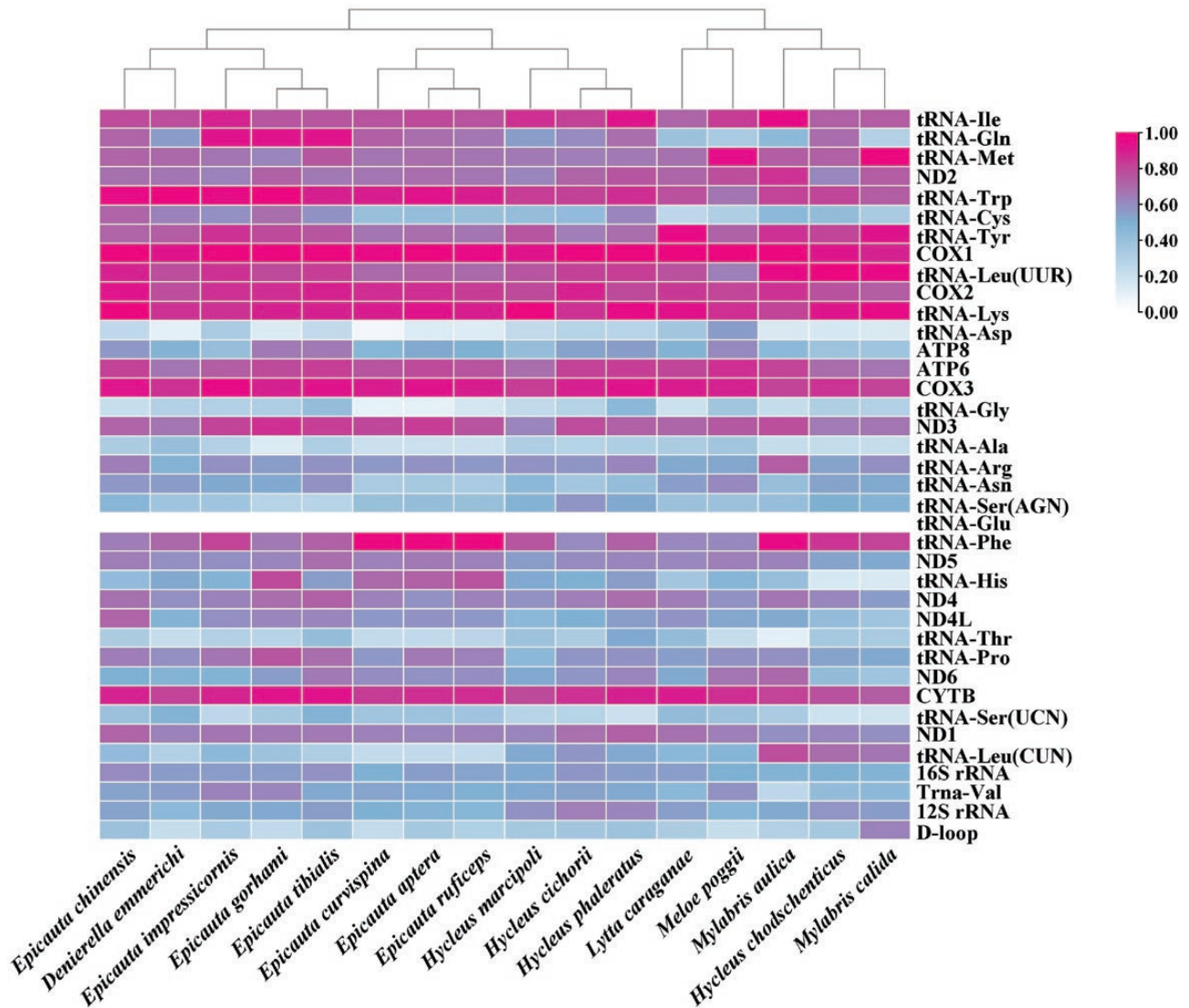

Fig. 3. Guanine–cytosine contents of each gene fragment in different populations.

The best partition schemes and substitution models for the maximum likelihood (ML) and Bayesian inference (BI) analyses of the datasets were estimated using PartitionFinder version 2.1.1 (Lanfear et al. 2017) with the greedy algorithm. ML analyses were performed using IQ-Tree version 1.6.3 (Nguyen et al. 2015), with 1,000 replicates of ultrafast likelihood bootstrapping (Minh et al. 2013) to obtain node support values. BI analysis was performed using MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001) with the following conditions: independent substitution model for each partition; four Markov chains; two independent runs each for 1,000,000 generations; and sampling every 1,000th generation, with the first 25% discarded as burn-in. The resulting phylogenetic trees were viewed and edited using FigTree version 1.4.2 (Mousavi et al. 2014).

Results

Genome Organization and Composition

The mitochondrial genome of *D. emmerichi* (GenBank no. MW476521; length: 15,702 bp), *E. curvispina* (GenBank no.

MW476523; length: 15,813 bp), and *M. poggii* (GenBank no. MW476520; length: 15,626 bp) is a double-stranded closed-loop molecule. The GC contents of the mitochondrial genome of *D. emmerichi*, *E. curvispina*, and *M. poggii* are 28, 33, and 36%, respectively (Fig. 1).

There are 12 gene overlaps in the mitochondrial genome of *D. emmerichi*, ranging in size from 1 to 10 bp. The longest gene overlap is between ATP6 and ATP8, with a length of 10 bp; the shortest four-gene overlaps are between tRNA-Gln and tRNA-Met, between tRNA-Trp and tRNA-Cys, between ATP6 and COX3, and between ND6 and CYTB, each with a length of 1 bp. There are five gene intervals, the longest being 17 bp between tRNA-Ser (UCN) and ND1 and the shortest being 2 bp between ND4L and tRNA-Thr and between tRNA-Pro and ND6 (Table 2). The gene spacing and gene overlap between adjacent genes in the mitochondrial genome of *E. curvispina* are consistent with those of *D. emmerichi*, whereas those of *M. poggii* differ from those of *E. curvispina* (Table 2). There are 13 gene overlaps in the mitochondrial genome of *M. poggii*; the longest gene

Table 1. Direction, position, and length of each gene segment of the three mitochondrial genomes

Gene	Strand			Location			Size (bp)		
	DS	EC	MG	DS	EC	MG	DS	EC	MG
tRNA-Ile	+	+	+	1–66	1–66	1–66	66	66	66
tRNA-Gln	–	–	–	64–132	64–132	64–132	69	69	69
tRNA–Met	+	+	+	132–200	132–200	132–200	69	69	69
ND2	+	+	+	201–1214	201–1,214	219–1,214	1,014	1,014	996
tRNA-Trp	+	+	+	1,213–1,278	1,213–1,279	1,213–1,278	66	67	66
tRNA-Cys	–	–	–	1,278–1,341	1,279–1,342	1,278–1,340	64	64	63
tRNA-Tyr	–	–	–	1,346–1,409	1,347–1,410	1,343–1,407	64	64	65
COX1	+	+	+	1,402–2,944	1,403–2,945	1,400–2,947	1,543	1,543	1,548
tRNA-Leu(UUR)	+	+	+	2,945–3,009	2,946–3,010	2,943–3,006	65	65	64
COX2	+	+	+	3,010–3,697	3,011–3,698	3,007–3,694	688	688	688
tRNA-Lys	+	+	+	3,698–3,767	3,699–3,768	3,695–3,765	70	70	71
tRNA-Asp	+	+	+	3,768–3,832	3,769–3,833	3,766–3,830	65	65	65
ATP8	+	+	+	3,842–3,994	3,843–3,995	3,831–3,992	153	153	162
ATP6	+	+	+	3,985–4,656	3,986–4,657	3,983–4,654	672	672	672
COX3	+	+	+	4,656–5,436	4,657–5,437	4,654–5,436	781	781	783
tRNA-Gly	+	+	+	5,437–5,499	5,438–5,500	5,440–5,502	63	63	63
ND3	+	+	+	5,497–5,851	5,498–5,852	5,503–5,856	355	355	354
tRNA-Ala	+	+	+	5,852–5,916	5,853–5,917	5,855–5,917	65	65	63
tRNA-Arg	+	+	+	5,917–5,979	5,918–5,980	5,918–5,981	63	63	64
tRNA-Asn	+	+	+	5,980–6,044	5,981–6,045	5,982–6,048	65	65	67
tRNA-Ser(AGN)	+	+	+	6,045–6,101	6,046–6,102	6,049–6,106	57	57	58
tRNA-Glu	+	+	+	6,102–6,163	6,103–6,164	6,107–6,167	62	62	61
tRNA-Phe	–	–	–	6,162–6,224	6,163–6,225	6,166–6,229	63	63	64
ND5	–	–	–	6,225–7,935	6,226–7,936	6,230–7,940	1,711	1,711	1,711
tRNA-His	–	–	–	7,936–7,999	7,937–8,000	7,941–8,004	64	64	64
ND4	–	–	–	8,000–9,332	8,001–9,333	8,005–9,337	1,333	1,333	1,333
ND4L	–	–	–	9,326–9,613	9,327–9,614	9,331–9,618	288	288	288
tRNA-Thr	+	+	+	9,616–9,679	9,617–9,680	9,621–9,683	64	64	63
tRNA-Pro	–	–	–	9,680–9,741	9,681–9,743	9,684–9,746	62	63	63
ND6	+	+	+	9,744–10,235	9,746–102,37	9,749–10,240	492	492	492
CYTB	+	+	+	10,235–11,374	10,237–11,376	10,240–11,379	1,140	1,140	1,140
tRNA-Ser(UCN)	+	+	+	11,373–11,439	11,375–11,441	11,378–11,445	67	67	68
ND1	–	–	–	11,457–12,407	11,459–12,409	11,463–12,413	951	951	951
tRNA-Leu(CUN)	–	–	–	12,408–12,471	12,410–12,473	12,414–12,477	64	64	64
16S rRNA	–	–	–	12,472–13,746	12,474–13,755	12,478–13,754	1,275	1,282	1,277
tRNA-Val	–	–	–	13,747–13,815	13,756–13,824	13,755–13,823	69	69	69
12S rRNA	–	–	–	13,816–14,604	13,825–14,613	13,824–14,614	789	789	791
D-loop				14,605–15,702	14,614–15,813	14,615–15,626	1,098	1,200	1,012

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

overlap is 10 bp between ATP6 and ATP8, and the shortest four-gene overlap is 1 bp, located between tRNA-Gln and tRNA-Met and between tRNA-Trp and tRNA-Trp (Table 2). There are six gene intervals between tRNA-Cys, ATP6, and COX3 and between ND6 and CYTB; the longest is 18 bp between tRNA-Met and ND2; the shortest is between tRNA-Cys and tRNA-Tyr and between ND4L and tRNA-Thr. The length between tRNA-Pro and ND6 is 2 bp (Table 2).

The mitochondrial genomes of *D. emmerichi*, *E. curvispina*, *M. poggii*, and other species of Meloidae (Du et al. 2016, 2017; Liu et al. 2020) contain 37 genes (13 PCGs, 22 tRNAs, and 2 rRNAs), of which 4 PCGs, 8 tRNA genes, and 2 rRNAs (12S rRNA and 16S rRNA) are encoded by the minor strand (N-strand), and the remaining are encoded by the major strand (J-strand; Fig. 2). On the basis of a comparison of the GC content between different gene segments of the known mitochondrial genome of the genus, it was concluded that the GC content of tRNA-Glu is the lowest among all segments, and the relatively highest content is COX1 (Fig. 3).
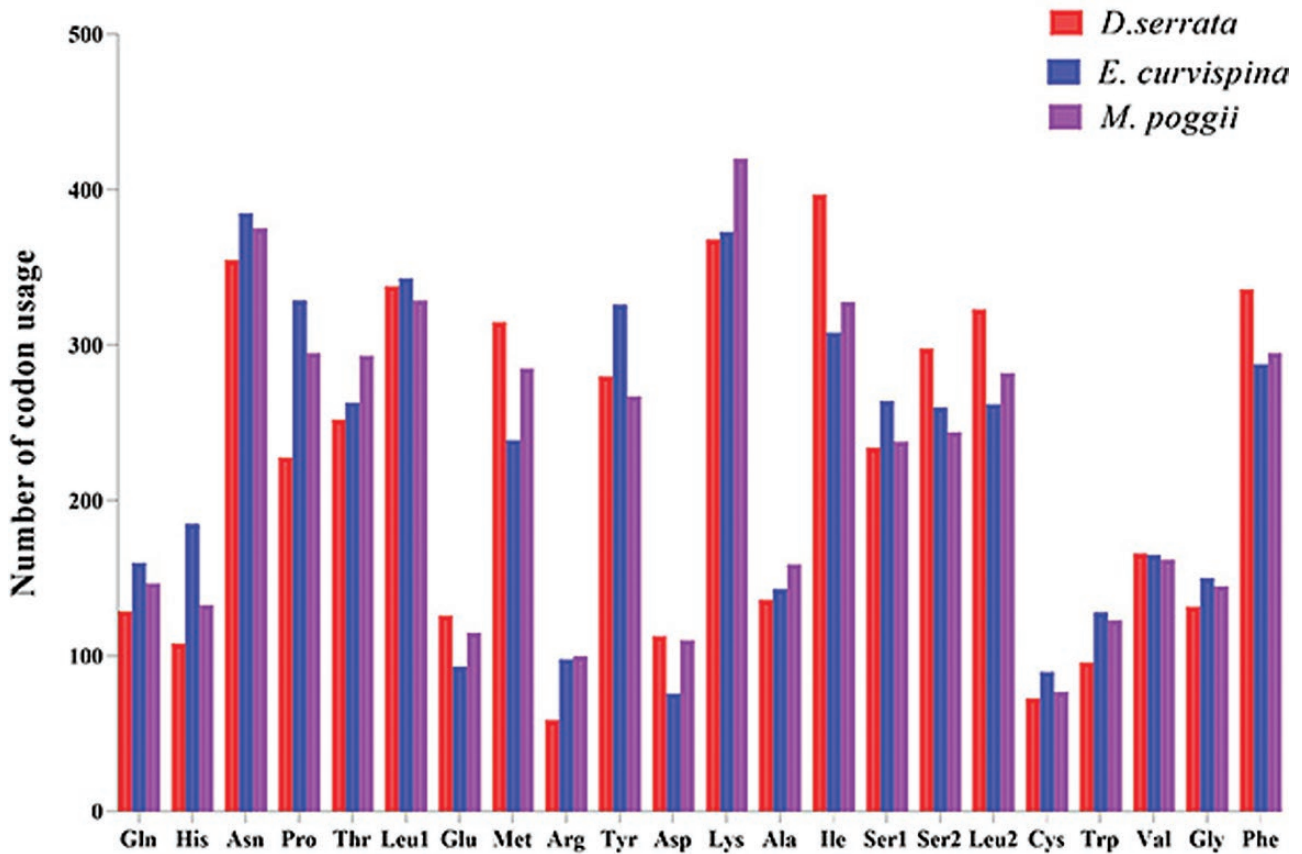
Protein-Coding Genes

Among the 13 PCGs of the mitochondrial genomes of *D. emmerichi*, *E. curvispina*, and *M. poggii*, 4 (ND1, ND4, ND4L, and ND5) are encoded on the J-strand and 9 (COX1, COX2, COX3, ATP6, ATP8, ND2, ND3, ND6, and CYTB) are encoded on the N-strand. The PCGs of *D. emmerichi* and *E. curvispina* are 11,121 bp in length, whereas those of *M. poggii* are 11,118 bp in length. The 13 PCGs of *D. emmerichi*, *E. curvispina*, and *M. poggii* all used ATN as the standard start codon and TAA, TAG, and T as the stop codons (Tables 1 and 2).

The amino acid usage and relative codon usage rate of the mitochondrial genomic PCGs were determined using RSCU analysis, which showed that among the 13 PCGs of *D. emmerichi*, *E. curvispina*, and *M. poggii*, the most common amino acid was Ile and the relatively least common amino acid was Cys (Fig. 4). In *D. emmerichi*, the most frequently used amino acid codon was UUA, and the least used was CGC; in both *E. curvispina* and *M. poggii*, the most frequently used amino acid codon was UCU, and the least used was ACG (Fig. 5).

Table 2. Anticodons of tRNAs, start/stop codons of protein-coding genes, and intergenic nucleotides of the three mitochondrial genomes

Gene	Anticodon			Start codon			Stop codon			Intergenic nucleotides		
	DS	EC	MG	DS	EC	MG	DS	EC	MG	DS	EC	MG
tRNA-Ile	GAT	GAT	GAT									
tRNA-Gln	TTG	TTG	TTG							−3	−3	−3
tRNA-Met	CAT	CAT	CAT							−1	−1	−1
ND2				ATA	ATA	ATT	TAA	TAA	TAA			18
tRNA-Trp	TCA	TCA	TCA							−2	−2	−2
tRNA-Cys	GCA	GCA	GCA							−1	−1	−1
tRNA-Tyr	GTA	GTA	GTA							4	4	2
COX1				ATT	ATT	ATT	T	T	TAA	−8	−8	−8
tRNA-Leu(UUR)	TAA	TAA	TAA									−5
COX2				ATA	ATA	ATA	T	T	T			
tRNA-Lys	CTT	CTT	CTT									
tRNA-Asp	GTC	GTC	GTC									
ATP8				ATA	ATA	ATT	TAA	TAA	TAA	9	9	
ATP6				ATG	ATG	ATG	TAA	TAA	TAA	−10	−10	−10
COX3				ATG	ATG	ATG	T	T	TAG	−1	−1	−1
tRNA-Gly	TCC	TCC	TCC									3
ND3				ATA	ATA	ATG	T	T	TAG	−3	−3	
tRNA-Ala	TGC	TGC	TGC									−2
tRNA-Arg	TCG	TCG	TCG									
tRNA-Asn	GTT	GTT	GTT									
tRNA-Ser(AGN)	TCT	TCT	TCT									
tRNA-Glu	TTC	TTC	TTC									
tRNA-Phe	GAA	GAA	GAA							−2	−2	−2
ND5				ATA	ATT	ATT	T	T	T			
tRNA-His	GTG	GTG	GTG									
ND4				ATG	ATG	ATG	T	T	T			
ND4L				ATG	ATG	ATG	TAA	TAA	TAA	−7	−7	−7
tRNA-Thr	TGT	TGT	TGT							2	2	2
tRNA-Pro	TGG	TGG	TGG									
ND6				ATT	ATT	ATT	TAA	TAA	TAA	2	2	2
CYTB				ATG	ATG	ATG	TAA	TAA	TAA	−1	−1	−1
tRNA-Ser(UCN)	TGA	TGA	TGA							−2	−2	−2
ND1				ATT	ATT	ATT	TAG	TAG	TAG	17	17	17
tRNA-Leu(CUN)	TAG	TAG	TAG									
16S rRNA												
tRNA-Val	TAC	TAC	TAC									
12S rRNA												
D-loop												

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

Fig. 4. Number of codon usages in the protein-coding genes of the mitochondrial genomes of *D. emmerichi*, *E. curvispina*, and *M. poggii*. Each amino acid is denoted as a three-letter symbol according to the three-letter amino acid codes established by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry and Molecular Biology joint commission.

Ribosomal RNA and Transfer RNA Genes

The mitochondrial genomes of *D. emmerichi*, *E. curvispina*, and *M. poggii* contain 22 typical tRNA genes, of which 14 are located on the J-strand and 8 are located on the N-strand (Table 1). The lengths of the tRNA genes of *D. emmerichi* and *E. curvispina* are between 57 (tRNA-Ser(AGN)) and 70 (tRNA-Lys) bp, whereas the lengths of the tRNA genes of *M. poggii* are between 58 (tRNA-Ser(AGN)) and 71 (tRNA-Lys) bp. The tRNA secondary structures of *D. emmerichi*, *E. curvispina*, and *M. poggii* were relatively conserved. Except for trnS1 (AGN), which loses the dihydrouridine arm, the other tRNAs can be folded into the classic clover secondary structure (Fig. 6).

In *D. emmerichi*, the length of the 16S rRNA is 1,275 bp and its GC content is 26%, and the length of the 12S rRNA is 789 bp and its GC content is 23% (Table 1). In *E. curvispina*, the length of the 16S rRNA is 1,282 bp and its GC content is 28%, and the length of the 12S rRNA is 789 bp and its GC content is 28% (Table 1). In *M. poggii*, the length of the 16S rRNA is 1,277 bp and its GC content is 26%, and the length of the 12S rRNA is 791 bp and its GC content is 25% (Table 1).

Phylogenetic Analysis

Based on the 13 PCGs and 13 PCGs + 2 rRNAs datasets of the mitochondrial genomes of 16 species belonging to 5 genera of Meloidae, a molecular phylogenetic tree was constructed using BI and ML (Figs. 7 and 8). The results of the BI and ML trees constructed using the two datasets are not very different, and both support the intergeneric phylogenetic relationship of ([*Epicauta* (*Denierella*) + (*Lytta* + *Meloe*)] + *Mylabris*); in particular, the support rate of (*Epicauta* [*Denierella*]) is the highest (Bayesian posterior probability [BPP] = 1, bootstrap percentage [BP] = 100).

Discussion

In this study, we determined the mitochondrial genome sequences of *D. emmerichi*, *E. curvispina*, and *M. poggii* and analyzed the structural characteristics of the mitochondrial genomes. We found that the GC content of the mitochondrial genome of *M. poggii* is the highest among the known mitochondrial genomes in Meloidae, which was 36% (Du et al. 2016, 2017; Liu et al. 2020; Fig. 4). The GC content of the mitochondrial genomes of *D. emmerichi*, *E. curvispina*, and *M. poggii* is lower than the AT content, which may be related to the frequency of amino acid codon usage in the mitochondrial genome. Among the known mitochondrial genomes of insects of Meloidae, the GC content differs among different gene segments is, although there is one common feature among different populations: the GC content of tRNA-Glu is the lowest, whereas the GC content of COX1 is relatively high. This finding will enrich further research on Meloidae mitochondrial genomics.

Denierella and *Epicauta* have very similar morphologies. The two genera only differ by the serration of the pretarsal claws in both sexes of *Denierella* (Batelka and Hájek 2015). However, a similar intrageneric variation in the claw structure exists in other large meloine genera (*Lytta*, *Lydomorphus*, *Mylabris*, etc.; Bologna and Pinto 2002). In the present study, the BI and ML trees that
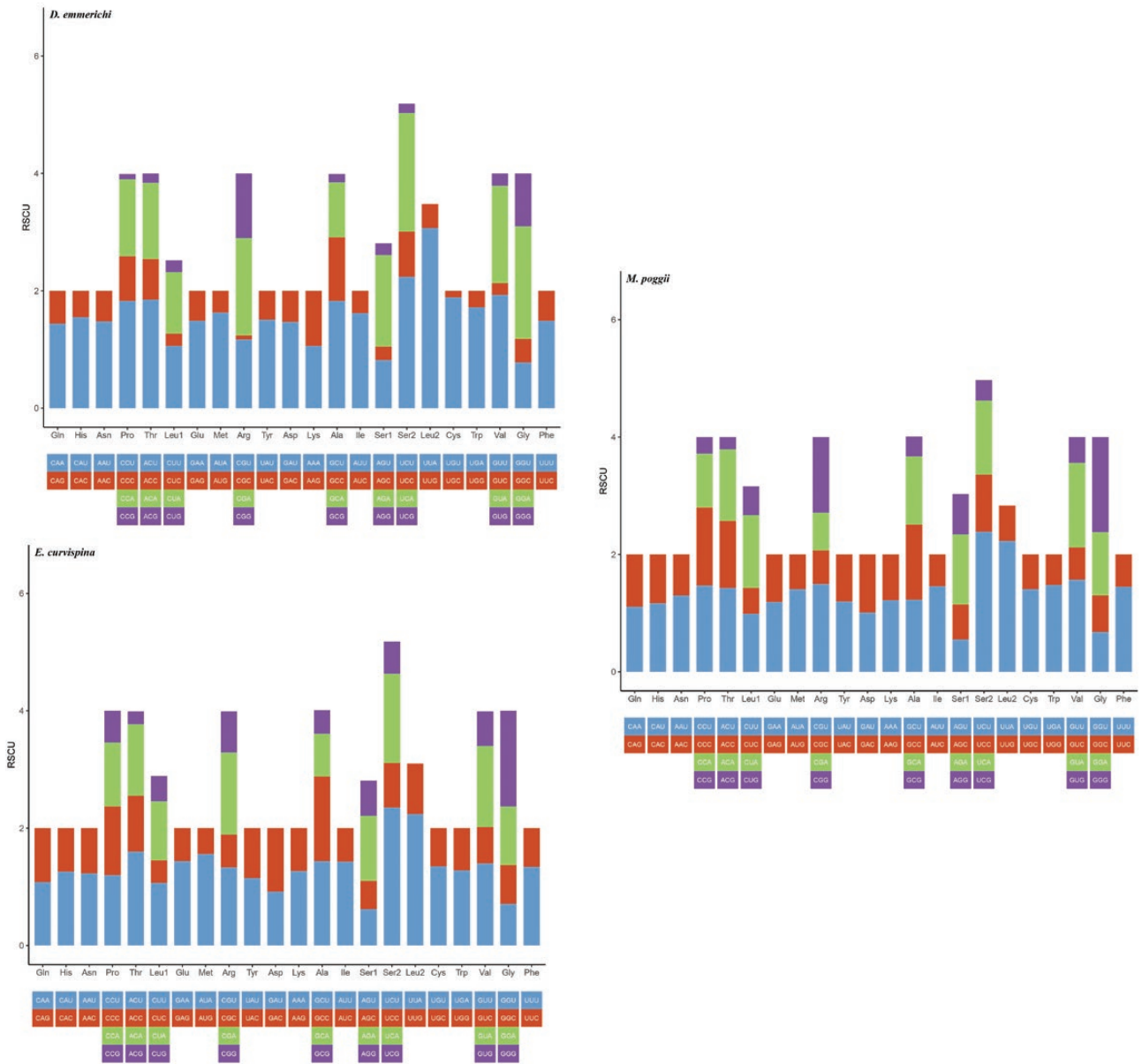
Fig. 5. Relative synonymous codon usage in the protein-coding genes of the mitochondrial genomes of *D. emmerichi*, *E. curvispina*, and *M. poggii*.
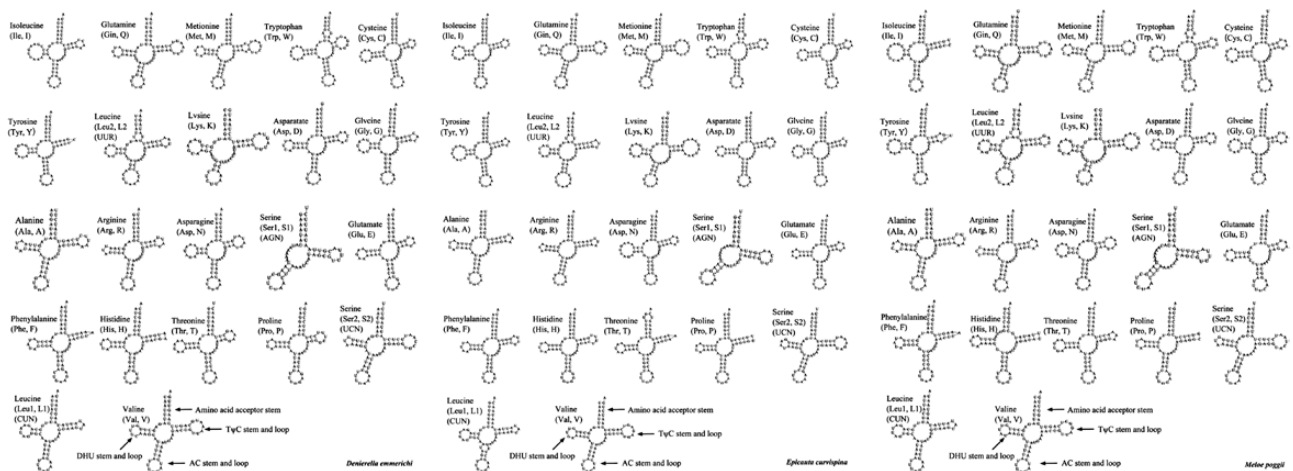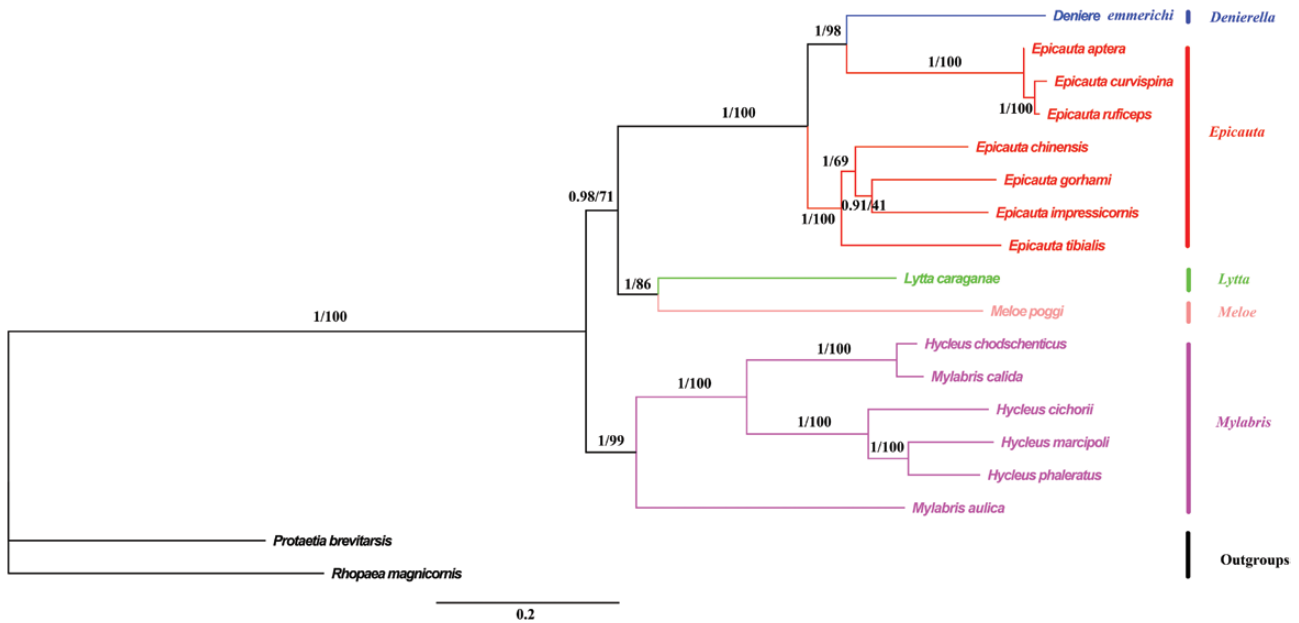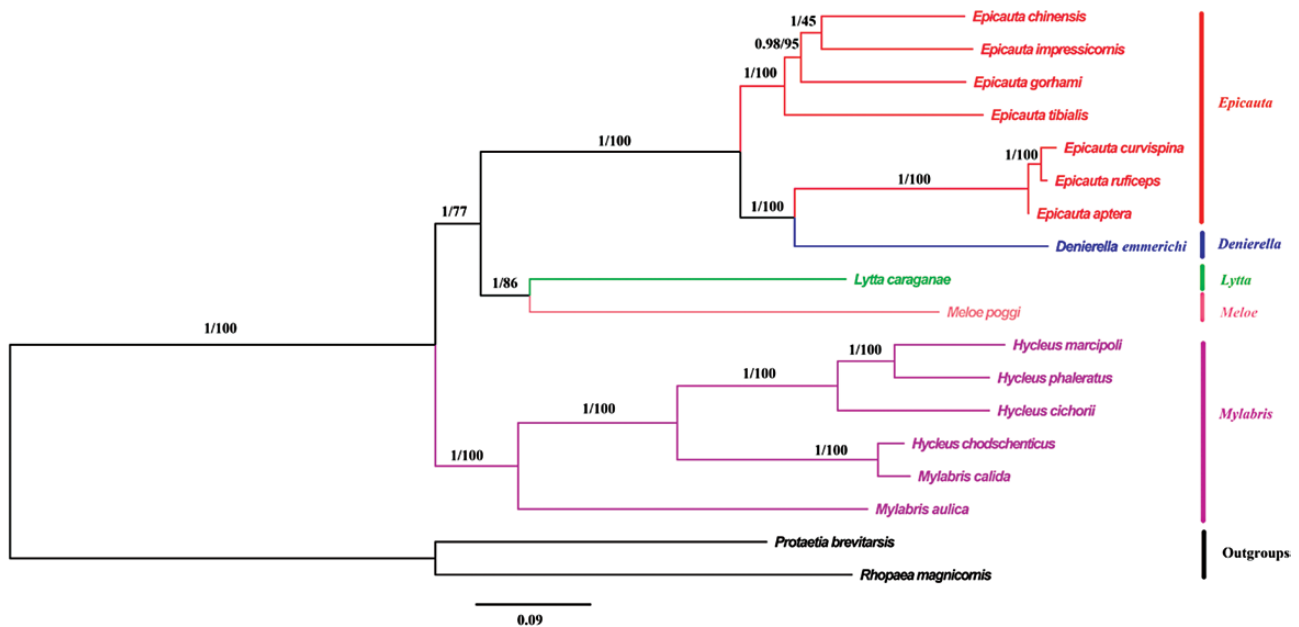

Fig. 6. Secondary structure of the tRNAs of *D. emmerichi*, *E. curvispina*, and *M. poggii* mitochondrial genomes.

Fig. 7. Phylogenetic trees of Meloidae inferred using MrBayes (Bayesian inference) and maximum likelihood analyses based on the nucleotide sequences of the 13 protein-coding genes. Bayesian posterior probabilities and bootstrap percentages are indicated on branches.

Fig. 8. Phylogenetic trees of Meloidae inferred using MrBayes (Bayesian inference) and maximum likelihood analyses based on the nucleotide sequences of the 13 protein-coding genes + 2 rRNAs. Bayesian posterior probabilities and bootstrap percentages are indicated on branches.

were constructed based on the 13 PCGs and 13 PCGs + 2 rRNAs datasets of the mitochondrial genomes of the Meloidae support *Epicauta* ([*D. emmerichi*, *E. curvispina*, *E. ruficeps*, *E. aptera*] + [*E chinensis*, *E. impressicornis*, *E. gorhami*, *E. tibialis*]), and the support rate is high (BPP = 1, BP = 100). Therefore, it is speculated that *D. emmerichi* belongs to the genus *Epicauta*, whereas *E. curvispina*, *E. ruficeps*, and *E. aptera* are closely related. As for the relationship between *Denierella* and *Epicauta*, further research and discussion are needed. Some researchers believe that *Denierella* is a subgenus of *Epicauta* (Bologna and Pinto 2002; Batelka and Hájek 2015; Pan and Ren 2020); the phylogenetic results in this study support this conclusion.

Acknowledgments

References Cited

Avise, J. C. 1994. Molecular markers, natural history and evolution. Springer, Boston, MA. pp. 60–91.

Batelka, J., and J. Hájek. 2015. New synonyms, combinations and faunistic records in the genus *Denierella* Kaszab (Coleoptera: Meloidae). Zootaxa 4000: 123–130.

Bologna, M. A., M. Oliverio, M. Pitzalis, and P. Mariottini. 2008. Phylogeny and evolutionary history of the blister beetles (Coleoptera, Meloidae). Mol. Phylogenet. Evol. 48: 679–693.

Bologna, M. A., and Pinto, J. D. 2002. The Old World genera of Meloidae (Coleoptera): a key and synopsis. J. Nat. Hist. 36: 2013–2102.

Boore, J. L. 1999. Animal mitochondrial genomes. Nucleic Acids Res. 27: 1767–1780.

Chen, C., H. Chen, Y. Zhang, H. R. Thomas, M. H. Frank, Y. He, and R. Xia. 2020. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol. Plant. 13: 1194–1202.

Du, C., S. He, X. Song, Q. Liao, X. Zhang, and B. Yue. 2016. The complete mitochondrial genome of *Epicauta chinensis* (Coleoptera: Meloidae) and phylogenetic analysis among Coleopteran insects. Gene 578: 274–280.

Du, C., L. F. Zhang, T. Lu, J. G. Ma, C. J. Zeng, B. S. Yue, and X. Y. Zhang. 2017. Mitochondrial genomes of blister beetles (Coleoptera, Meloidae) and two large intergenic spacers in *Hycleus* genera. BMC Genomics 18: 698.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874.

Lanfear, R., P. B. Frandsen, A. M. Wright, T. Senfeld, and B. Calcott. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34: 772–773.

Li, X. M., Li, J., and Pan, Z. 2020. New species and new faunistic records of the family Meloidae Gyllenhal, 1810 (Coleoptera: Tenebrionoidea) from China, with a list of meloid species from Xinjiang. J. Asia Pac. Entomol. 23: 1144–1150.

Liu, Y. Y., G. Y. Li, L. Yang, H. Chi, and X. S. Chen. 2018. Demography and mass rearing of the medicinal blister beetle *Epicauta impressicornis* (Pic) (Coleoptera: Meloidae) at different temperatures. J. Econ. Entomol. 111: 2364–2374.

Liu, Y. Y., Pan, Z., and Chen, X. S. 2016. Comparative morphological study on five medicinal *Epicauta* species from Guizhou province. Sichuan J. Zool. 35: 84–92.

Liu, Y. Y., Zhou, Z. C., and Chen, X. S. 2020. Characterization of the complete mitochondrial genome of *Epicauta impressicornis* (Coleoptera: Meloidae) and its phylogenetic implications for the infraorder Cucujiformia. J. Insect Sci. 20: 1–10.

Lowe, T. M., and S. R. Eddy. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25: 955–964.

Meng, G., Y. Li, C. Yang, and S. Liu. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Res. 47: e63.

Minh, B. Q., M. A. Nguyen, and A. von Haeseler. 2013. Ultrafast approximation for phylogenetic bootstrap. Mol. Biol. Evol. 30: 1188–1195.

Mousavi, S. A., J. Österman, N. Wahlberg, X. Nesme, C. Lavire, L. Vial, L. Paulin, P. de Lajudie, and K. Lindström. 2014. Phylogeny of the Rhizobium–Allorhizobium–Agrobacterium clade supports the delineation of *Neorhizobium* gen. nov. Syst. Appl. Microbiol. 37: 208–215.

Nguyen, L. T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32: 268–274.

Okano, R., Hiroyuki, Y., and Yano, S. 2015. Courtship behavior of three *Meloe* species (Coleoptera, Meloidae). SAYABANE 19: 45–49.

Pan, Z., and M. A. Bologna. 2021. Morphological revision of the Palaearctic species of the nominate subgenus *Meloe* Linnaeus, 1758 (Coleoptera, Meloidae), with description of ten new species. Zootaxa 5007: 1–74.

Pan, Z., and Ren, G. D. 2020. New synonyms, combinations and status in the Chinese species of the family Meloidae Gyllenhal, 1810 (Coleoptera: Tenebrionoidea) with additional faunistic records. Zootaxa 4820: 260–286.

Pan, Z., and Ren, G. D. 2018. Taxonomic revision of the subfamily Meloinae (Coleoptera: Meloidae) from Xizang, China, with description of a new species. Zool. Syst. 43: 66–88.

Shintani, Y., Hirose, Y., and Terao, M. 2010. Effects of temperature, photoperiod and soil humidity on induction of pseudopupal diapause in the bean blister beetle *Epicauta gorhami*. Physiol. Entomol. 36: 14–20.

Shintani, Y., M. Terao, and S. Tanaka. 2017. Adaptive significance of precocious pupation in the bean blister beetle, *Epicauta gorhami* (Coleoptera: Meloidae), a hypermetamorphic insect. J. Insect Physiol. 99: 107–112.

Terao, M., Y. Hirose, and Y. Shintani. 2012. Effects of temperature and photoperiod on termination of pseudopupal diapause in the bean blister beetle, *Epicauta gorhami*. J. Insect Physiol. 58: 737–742.

Terao, M., Hirose, Y., and Shintani, Y. 2015. Food-availability dependent premature metamorphosis in the bean blister beetle, *Epicauta gorhami* (Coleoptera: Meloidae), a hypermetamorphic insect that feeds on grasshopper eggs in the larval stage. Entomol. Sci. 18: 85–93.

Vaidya, G., Lohman, D. J., and Meier, R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27: 171–180.

Wolstenholme, D. R. 1992. Animal mitochondrial DNA: structure and evolution. Int. Rev. Cytol. 141: 173–216.

Yang, Y. X., and Ren, G. D. 2007. Review of Chinese species of the genus *Denierella* Kaszab with a key to the worldwide species (Coleoptera, Meloidae). Zootaxa 1642: 53–59.

Zhang, D., F. Gao, I. Jakovlić, H. Zou, J. Zhang, W. X. Li, and G. T. Wang. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol. Ecol. Resour. 20: 348–355.

Zhang, D. X., and Hewitt, G. M. 1997. Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. Biochem. Syst. Ecol. 25: 99–120.