First Report of Complete Mitochondrial Genome in the Tribes Coomaniellini and Dicercini (Coleoptera: Buprestidae) and Phylogenetic Implications

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Abstract: The complete mitochondrial genomes (mitogenomes) of the tribes Coomaniellini and Dicercini were sequenced and described in this study, including Coomaniella copipes (16,196 bp), Coomaniella dentata (16,179 bp), and Dicerca corrugata (16,276 bp). These complete mitogenomes are very similar in length and encoded 37 typical mitochondrial genes, including 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs) and 13 protein-coding genes (PCGs). Most of PCGs had typical ATN start codons and terminated with TAR. Among these mitogenomes, Leu2 (L2), Ile (I), Ser2 (S2), and Phe (F) were the four most frequently encoded amino acids. Moreover, phylogenetic analyses were performed based on three kinds of nucleotide matrixes (13 PCGs, 2 rRNAs, and 13 PCGs + 2 rRNAs) among the available sequenced species of the family Buprestidae using Bayesian inference and Maximum-likelihood methods. The results showed that a Chrysochroninae species interspersed in Buprestinae, and Coomaniellini is more closely related to Dicercini than Melanophilini. Moreover, the clade of Buprestidae was well separated from outgroups and the monophyly of Agrilinae is confirmed again. Our whole mitogenome phylogenetic results support that the genus Dicerca can be transferred from Chrysochroinae to Buprestinae; whether Dicercini can be completely transferred remains to be further verified after enriching samples. Our results have produced new complete mitogenomic data, which will provide information for future phylogenetic and taxonomic research.

Keywords: Buprestidae; Coomaniellini; Dicercini; mitogenome; phylogenetic analysis

1. Introduction

The family Buprestidae is one of the largest families in Coleoptera, which comprises six subfamilies, 521 genera, and more than 15,000 species [1,2], widely distributed in the world. Some are important forestry and agricultural pests that threaten forest ecosystems and damage economical crops, especially in the subfamilies Agrilinae [3–7] and Buprestinae [8,9].

Although some taxonomists have made various contributions to the Buprestidae classification based on morphological characteristics [1,2,10–14], the phylogenetic relationships in subfamilies and tribes are still unresolved completely, such as: subfamilies Buprestinae and Chrysochroinae, tribes Tracheini, Agrilini, Coraebini, and Melanophilini, etc. Moreover, there is very little work on molecular phylogeny of higher taxa. Evans et al. [15] made the first large-scale molecular phylogeny of Buprestidae, results showed that the subfamilies Chrysochroinae and Buprestinae were polyphyletic, while the Agrilinae, Julodinae, and Galbellinae were monophyletic; the tribes Agrilini, Coraebini, and Tracheini were polyphyletic. Recently, molecular species delimitation was applied to Buprestidae. This method was used to analyze the Chrysobothris femorata species group by Hansen et al. [16], the Agrilus species by Pentinsaari et al. [17], Pellegrino et al. [18], and Kelnarova et al. [19], their results showed that DNA barcoding is a powerful species identification tool.
Additionally, the phylogenetic relationships of Melanophilini, Coomaniellini, and Dicercini remain unsettled. The tribe Dicercini comprises 29 genera widely distributed in Asia, Europe, Africa, South America, and North America [1,2]. One of the authors found the Dicera species inhabit in plants Pinus sp. the biological characteristics of which are less known. The monogenic tribe Coomaniellini was proposed by Bily [20]. The adults of Coomaniella were usually found on leaves of Anacardiaceae [21,22]. All the known species of Coomaniella are distributed in South and Southeast Asia. The tribe Melanophilini comprises seven genera: juniperella, Melanophila, Phaenops, Trachypteris, Xenomelanopa, Merimna, and Cromophila [1,2], widely distributed in Africa, Europe, Asia, North America, and South America. To date, the position of these tribes had yet been resolved in taxonomical classification.

In the past two decades, mitogenome emerged as an important source on phylogenetic analysis [23–27], evolution strategies [28–31], and genetic diversity [32–34] and species delimitation [35]. In 2009, the first mitogenome in Buprestidae (Chrysosochra fulgidaissima) was published [36]. Xiao et al. [37] reported the mitogenome of Trachys auricollis and carried out a phylogenetic analysis of Elateriformia, which showed that the Buprestoidea is a sister group to Byrrhoidae. Sun et al. [38] also performed a phylogenetic analysis of suborder Polyphaga, and the results showed that the Buprestidae is a sister group to other Polyphaga infraorders. To date, 18 complete mitogenome sequences have been reported (including three species in this study), details are shown in Table 1.

### Table 1. Taxonomy, GenBank accession numbers, and related information on the mitochondrial genomes used for the phylogenetic analysis.

| No. | Family/Subfamily | Taxa | Accession No. | Genome Size (bp) | A + T% | AT-Skew | GC-Skew | Location/References |
|-----|------------------|------|---------------|------------------|--------|---------|---------|---------------------|
| 1   | Buprestinae      | Melanophila acuminata | MW287594 | 15,853 | 75.66 | 0.02 | −0.25 | [39]               |
| 2   | Buprestinae      | Anthacus chinensis | MW99326 | 15,881 | 73.61 | 0.09 | −0.29 | [40]               |
| 3   | Buprestinae      | Coomaniella copipes | OL694145 | 16,196 | 74.47 | 0.03 | −0.24 | This study          |
| 4   | Buprestinae      | Coomaniella dentata | OL694144 | 16,179 | 76.59 | 0.01 | −0.21 | This study          |
| 5   | Chrysochroinae   | Dicerca corrugata | OL753086 | 16,276 | 71.76 | 0.09 | −0.21 | This study          |
| 6   | Chrysochroinae   | Chrysoschroa fulgidissima | EJ826483 | 15,992 | 69.92 | 0.15 | −0.24 | [36]               |
| 7   | Agrilinae        | Corax dermatus | OK189521 | 15,499 | 68.42 | 0.12 | −0.25 | [41]               |
| 8   | Agrilinae        | Corax clavatus | OK189520 | 15,514 | 69.27 | 0.11 | −0.25 | [41]               |
| 9   | Agrilinae        | Melitius sinae | OK189522 | 16,108 | 72.42 | 0.11 | −0.22 | [41]               |
| 10  | Agrilinae        | Sambus fمور | OK394949 | 15,367 | 73.23 | 0.12 | −0.18 | [41]               |
| 11  | Agrilinae        | Agrilus siccatus | OK189519 | 16,521 | 71.73 | 0.12 | −0.21 | [41]               |
| 12  | Agrilinae        | Agrilus planipennis | KT363584 | 15,942 | 71.90 | 0.12 | −0.24 | [42]               |
| 13  | Agrilinae        | Agrilus malv | MN948990 | 16,204 | 74.46 | 0.08 | −0.18 | [38]               |
| 14  | Agrilinae        | Corax carinifrons | MK913589 | 15,686 | 69.79 | 0.12 | −0.18 | [43]               |
| 15  | Agrilinae        | Trachys auricollis | MH163286 | 16,429 | 71.05 | 0.10 | −0.20 | [37]               |
| 16  | Agrilinae        | Trachys troglodytiformis | KW973567 | 16,316 | 74.62 | 0.10 | −0.19 | unpublished        |
| 17  | Agrilinae        | Trachys variolaris | MN178497 | 16,271 | 72.11 | 0.11 | −0.21 | [44]               |
| 18  | Polycinetinae    | Acmaeodera sp. | FJ613420 | 16,217 | 68.41 | 0.11 | −0.25 | [45]               |
| 19  | Heteroceridae    | Heterocerus parallelus | KW973567 | 15,845 | 74.03 | 0.13 | −0.24 | unpublished        |
| 20  | Dryopidae        | Dryops ernesti | KW973567 | 15,672 | 72.98 | 0.07 | −0.23 | unpublished        |

In this study, the mitogenome sequences of Dicerca corrugata, Coomaniella copipes, and C. dentata are determined and annotated, which are the first complete mitogenome sequences reported in the tribes Coomaniellini and Dicercini. And the mitogenome of Melanophila acuminata was reported by Peng et al. [39], which belongs to the tribe Melanophilini. A preliminary phylogenetic analysis was undertaken to validate the phylogenetic position of the tribes Melanophilini, Coomaniellini, and Dicercini based on the mitogenomes. The results of this study may provide new data for phylogenetic studies of the Buprestidae and expand our knowledge of the mitochondrial genomic features of Buprestidae and the taxonomy within the family Buprestidae.

## 2. Materials and Methods

### 2.1. Sampling and DNA Extraction

The specimens of Dicerca corrugata were collected from plant Pinus kwangtungensis in Rufeng, Nanling National Forest Park, Shaoguan City, Guangdong Province, China (24.898625° N, 113.24.01945° E, alt. 1542 m) on 23 May 2021. The specimens of Coomaniella
dentata and C. copipes were collected from Guitian Village, Dayao Mountains, Jinxiu County, Guangxi Zhuang Autonomous Region, China (24.07335° N, 110.16771° E, alt. 800–1000 m), on 20 April 2021. These specimens were preserved in 95% ethanol at −24 °C for the long-term storage in the specimen collection room at China West Normal University. The total genomic DNA was extracted from muscle tissue of individual specimens using the Ezup Column Animal Genomic DNA Purification Kit (Shanghai, China) following the manufacturer’s instructions. For sequencing, the extracted DNA was stored at −24 °C.

2.2. Sequence Assembly, Annotation, and Analysis

Next-generation sequencing and assembly were performed by Beijing Aoweisen Gene Technology Co. Ltd. (Beijing, China) to obtain the complete mitogenome sequences of the three Buprestidae species. A whole genome shotgun strategy was used based on the Illumina HiSeq platform when the total genome DNA was quantified. The sequencing with a strategy of 150 bp paired-end reads. The assembly method of Hahn et al. [46] was used. The complete mitogenome sequences of three Buprestidae species were annotated using Geneious 11.0.2 [47] based on the invertebrate genetic code. The 22 tRNA genes were re-verified using MITOS webserver [48] based on the invertebrate mitogenetic code. The tRNA secondary structures were predicted using tRNAscan-SE [49]. The mitogenome maps were drawn using Organellar Genome DRAW [50]. The base composition and relative synonymous codon usage (RSCU) values were calculated using MEGA 7.0 [51]. Strand asymmetry was calculated in terms of formulae: AT-skew = (A − T)/(A + T) and GC-skew = (G − C)/(G + C) [52]. Nucleotide diversity (Pi), non-synonymous substitutions (Ka), and synonymous substitutions (Ks) of 13 PCGs were calculated using DnaSP v 5 [53].

2.3. Phylogenetic Analysis

A total of 18 buprestid complete mitogenomes (Table 1), including three newly sequenced species were used to construct the phylogenetic tree of 18 species from 11 genera belonging to 4 subfamilies of Buprestidae, with Heterocerus parallelus (Heteroceridae) and Dryops ernesti (Dryopidae) as outgroups based on the phylogenetic analyses of Polyphaga [37]. Three datasets (13 PCGs, 2 rRNAs, and 13 PCGs + 2 rRNAs) were used to construct the phylogenetic trees using PhyloSuite v 1.2.2 [54] based on the Maximum likelihood (ML) and Bayesian inference (BI) methods using different best-fit substitution models. The sequences were aligned using ClustalW [55] and trimmed by trimAl v 1.2 [56]. The best-fit models used in ML and BI analyses were calculated with ModelFinder [57]. The phylogenetic trees were reconstructed using IQ-tree v 1.6.8 [58] and MrBayes v 3.2.6 program [59] based on the ML and BI methods, respectively. Maximum likelihood analyses were run with 1000 ultrafast bootstrap and 1000 SH-aLRT replicates to estimate node reliability. Bayesian analyses were run with two independent chains spanning 2,000,000 generations, four Markov chains, sampling at every 100 generations, and with a burn-in of 25%. The phylogenetic trees were visualized and edited using FigTree v1.4 [60].

3. Results and Discussion

3.1. Genome Organization and Base Composition

We sequenced and annotated the complete mitogenomes of the buprestid species C. copipes (GenBank accession no. OL694145; SRA accession no. SRR19612349; length: 16,196 bp), C. dentata (OL694144; SRR19612367; 16,179 bp), and D. corrugata (OL753086; SRR19629749; 16,276 bp). Overall, these three mitogenomes have the same composition, consisting of 37 coding genes (13 PCGs, 22 tRNA, and two rRNA) and a non-coding A + T-rich region. Four PCGs (nad5, nad4, nad4l, and nad1), eight tRNAs (trnQ, trnC, trnY, trnF, trnH, trnP, trnL1, and trnV), and two rRNAs are encoded on the N-strand, while the other 23 genes (9 PCGs, 14 tRNAs) and the A + T-rich region are encoded on the J-strand (Figures 1 and S1).
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Figure 1. Gene maps of the mitogenomes of three Buprestidae species sequenced in this study.

The three complete mitogenomes had high A + T contents of 71.76–76.59% and had a positive AT-skews and negative GC-skews. The overall AT-skews in the three entire mitogenomes were 0.03, 0.01, and 0.09, respectively (Table 1). The total length of 13 overlapping regions in the whole mitogenome of \textit{C. copipes} is 37 bp, while \textit{C. dentata} has 10 overlapping regions with a length of 33 bp, and \textit{D. corrugata} has 12 overlapping regions, the length is 38 bp (Table 2). However, the longest overlapping regions of all three species are 8 bp, located between \textit{trnW} and \textit{trnC}, which is consistent with the results of some other studies on Buprestidae species with only slight differences in length, such as the longest overlapping region of 9 bp for \textit{Chrysochroa fulgidissima} [36] and 8 bp for \textit{Trachys troglodytiformis} [37]. In these three species, there is a 7 bp overlapping region between \textit{atp8} and \textit{atp6}, \textit{nad4} and \textit{nad4l}, which is often reported in the mitochondrial genome of insects.

The gene arrangement, nucleotide composition, and codon usage of these three mitogenomes were consistent with other buprestid species [36–40,42–44]. The same order of genes is not enough to unify taxa, but genes with the same rearrangement can be an effective marker of common ancestor [61]. The arrangement and rearrangement of genes may be used as a very favorable means to assist classification and infer ancient evolutionary relationships, since rearrangement is usually a unique and rare event [62]. The rearrangement phenomenon has been found in some studies [63–66], but it has not been detected in these three species.
Table 2. The three newly annotated Buprestidae mitogenomes.

| Gene | Strand | Position | Codons | Anticodon | IGN |
|------|--------|----------|--------|-----------|-----|
|      | From   | To       | Start  | Stop      |     |
| nad2 | J       | 199/201/201 | 1221/1223/1223 | ATT/ATT/ATT | TAA/TAA/TAA | –2/–2/–2 |
| cox1 | J       | 1405/1407/1405 | 2935/2937/2935 | –/-/- | T/T/T | 0/0/0 |
| cox2 | J       | 3001/3003/3001 | 3685/3684/3682 | ATA/ATA/ATA | T/T/T | 0/0/0 |
| atp8 | J       | 3822/3824/3817 | 3977/3979/3972 | ATT/ATC/ATT | TAA/TAA/TAA | –7/–7/–7 |
| atp6 | J       | 3971/3973/3966 | 4645/4647/4640 | ATG/ATG/ATG | TAA/TAA/TAA | –1/–1/–1 |
| cox3 | J       | 4645/4647/4640 | 5431/5433/5426 | ATG/ATG/ATG | T/T/T | 0/0/0 |
| nad3 | J       | 5494/5496/5490 | 5847/5849/5843 | ATT/ATT/ATT | TAG/TAG/TAG | –2/–1/–2 |
| nad5 | N       | 6231/6229/6227 | 7950/7948/7946 | ATT/ATT/ATT | T/T/T | 0/0/0 |
| nad4 | N       | 8016/8013/8012 | 9351/9348/9347 | ATG/ATG/ATG | T/T/T | –7/–7/–7 |
| nadH | N       | 9345/9342/9341 | 9629/9626/9631 | ATG/ATG/ATG | TAA/TAA/TAA | 2/2/2 |
| nad6 | J       | 9760/9757/9763 | 10,269/10,266/10,263 | ATT/ATT/ATT | TAA/TAA/TAA | –1/–1/–1 |
| cyt b | J     | 10,269/10,266/10,263 | 11,411/11,408/11,405 | ATG/ATG/ATG | TAG/TAG/TAG | –2/–2/–2 |
| nad1 | N       | 11,493/12,485/11,501 | 12,446/12,441/12,451 | TTG/TTG/TTG | TAA/TAA/TAG | 1/1/1 |
| trnl | J       | 1/1/1 | 64/65/65 | GAT | –3/–3/–3 |
| trnQ | N       | 62/63/63 | 130/131/131 | TGG | –1/0/0 |
| trnM | J       | 130/132/132 | 198/200/200 | CAT | 0/0/0 |
| trnW | J       | 1220/1222/1222 | 1284/1286/1287 | TCA | –8/–8/–8 |
| trnC | N       | 1277/1279/1280 | 1337/1339/1341 | GCA | 0/0/0 |
| trnY | N       | 1338/1340/1342 | 1403/1405/1403 | GGA | 0/0/0 |
| trnL2 | J     | 2936/2938/2936 | 3000/3002/3000 | TAA | 0/0/0 |
| trnK | J       | 3686/3685/3683 | 3755/3754/3752 | CTT | 0/0/0 |
| trnD | J       | 5756/3755/3753 | 3820/3823/3816 | GTC | 1/0/0 |
| trnG | J       | 5432/5434/5427 | 5493/5495/5489 | TTC | 0/0/0 |
| trnA | J       | 5846/5849/5842 | 5908/5910/5904 | TGC | –1/0/–1 |
| trnR | J       | 5908/5911/5904 | 5971/5970/5970 | TCG | –1/0/–3 |
| trnN | J       | 5971/5971/5968 | 6037/6035/6032 | GTG | 0/0/0 |
| trnS1 | J     | 6038/6036/6033 | 6104/6101/6099 | TCT | 0/0/0 |
| trnE | J       | 6105/6102/6100 | 6168/6165/6163 | TTC | –1/1/–1 |
| trnF | N       | 6168/6165/6163 | 6202/6226/6226 | GAA | 0/0/0 |
| trnH | N       | 7951/7949/7947 | 8015/8012/8011 | GTG | 0/0/0 |
| trnT | J       | 9632/9629/9634 | 9694/9691/9696 | TGT | 0/0/0 |
| trnP | N       | 9695/9692/9697 | 9758/9755/9761 | TGG | 1/1/1 |
| trnL3 | N     | 12,448/12,443/12,453 | 12,510/12,505/12,517 | TGA | 0/0/0 |
| trnV | N       | 13,800/13,789/13,800 | 13,867/13,856/13,869 | TAG | 0/0/0 |
| rrmL | N      | 12,511/12,506/12,518 | 13,799/13,788/13,799 | TAC | 0/0/0 |
| rrmS | N      | 13,868/13,857/13,870 | 14,582/14,569/14,608 | TAC | 0/0/0 |
| A + T-rich region J | 14,583/14,570/14,609 | 16,196/16,179/16,276 | 0/0/0 |

The order of these three species in the table is as follows: C. copipes, C. dentata, and Dicerca corrugata. IGN = intergenic nucleotides; – not determined.

3.2. Protein-Coding Regions, Codon Usage and Nucleotide Diversity

In these three mitogenomes, the regions of PCGs were 11,159 bp (C. copipes), 11,159 bp (C. dentata) and 11,150 bp (D. corrugata) in size, accounting for 75.41–78.66% of the entire mitogenome. The mitogenomes can be converted into 3707–3710 amino acid-coding codons, excluding stop codons (29 bp). We found the atp8 and nad5 to be the smallest (156 bp) and the largest (1720 bp) genes, respectively, which is similar to the other buprestid mitogenomes. The majority of PCGs directly use ATN (ATA/ATT/ATG/ATC) as the start codon, the exception is nad1 gene, which starts with TTG. The unusual start codon of the nad1 gene can be found in the mitogenomes of some other insects, such as Trachys auricollis (TTG) and Liriomyza trifolii (GTG) [38,67]. The start codon of cox1 gene has not been determined in this study; nevertheless, it can be found in some reports that cox1 is always nonstandard, for example, CGA in Laelia suffuse [64], AAA in Tribolium castaneum [68], or even ATCA in Liriomyza trifolii [67]. Moreover, except for five PCGs (cox1, cox2, cox3, nad5, and nad4) with an incomplete stop codon T-, the other eight PCGs have complete stop codons (TAA/TAG). The stop codon T- of the five genes was completed by the addition of 3′ A residues to the mRNA [69,70]. This phenomenon of generating TAA ends by post-transcriptional polyadenylation has been seen in many studies [36,37,65].
A summary of the number of amino acids in annotated PCGs (Figure 2A) is presented, along with the percentage of the top 11 amino acids with higher numbers (Figure 2B) and relative synonymous codon usage (Figure 3; Tables S1–S3). We can conclude that the overall codon usage among the newly sequenced species was similar, with L2, I, S2, and F being the four most frequently used amino acids, and TTA (L2), ATT (I), TTT (F), and ATA (M) are the most frequently used codons.

Figure 2. Numbers of different amino acids in the mitogenomes of the three Buprestidae species (A) and the percentage of top 11 amino acids with higher number (B); the stop codon is not included. CC: Coomaniella copipes, CD: Coomaniella dentata, DC: Dicerca corrugata.

The nucleotide diversity (Pi) of the 13 PCGs among three newly sequenced species is provided (Figure 4), which ranged from 0.159 to 0.297. In these genes, atp8 (Pi = 0.297) presented the highest variability, followed by nad6 (Pi = 0.292), nad2 (Pi = 0.264), and nad3 (Pi = 0.261); cox1 (Pi = 0.159) exhibited the lowest variability. For the study of insect taxonomy and the analysis of species evolution, the mitochondrial gene cox1 was primarily used because it is relatively conservative (Table S4). The ratio of Ka/Ks for each gene of the 13 PCGs was calculated (Figure 5). The value of nad1 gene is obviously higher than others, which indicates that the nad1 gene has a relatively higher evolutionary rate. Meanwhile, the Ka/Ks ratios of the other 12 PCGs were all significantly less than 1, and the value of the cox1 gene is the lowest (Ka/Ks = 0.05). Indeed, cox1 shows the lowest Ka/Ks ratio in almost all insects, that is, a relatively low variation rate. Moreover, Xiao et al. [37] indicated that not only insects but almost all animals have the lowest Ka/Ks ratio for cox1. This indicates that the gene was subjected to the highest purifying selection [71]. The genes with the lowest and highest Ka were cox1 (0.044) and atp8 (0.286), respectively, while the Ks were nad1 (0.189) and nad3 (1.553) were the lowest and highest genes.
Figure 3. Relative synonymous codon usage (RSCU) of the mitogenomes of the three Buprestidae species; the stop codon is not included. Coomaniella copipes (A), Coomaniella dentata (B), Dicerca corrugata (C).

Figure 4. Nucleotide diversity (Pi) of 13 PCGs in three newly sequenced buprestid mitogenomes.

Figure 5. The ratio of Ka/Ks of 13 PCGs in three newly sequenced buprestid mitogenomes.

3.3. Ribosomal and Transfer RNA Genes

The lengths of \textit{rrnL} genes ranged from 1282 bp (\textit{D. corrugata}) to 1289 bp (\textit{C. copipes}), whereas those of \textit{rrnS} ranged from 713 bp (\textit{C. dentata}) to 739 bp (\textit{D. corrugata}). The AT content of the rRNA genes ranged from 75.41% to 78.66% (Table 3). These rRNA genes are located between \textit{trnL1} and A + T-rich region and separated by \textit{trnV}. There are no gaps between the rRNA genes. The total lengths of the 22 tRNA genes ranged from 1429 bp (\textit{C. dentata}) to 1441 bp (\textit{D. corrugata}), while the length of individual tRNA genes generally ranged from 60 to 70 bp, among which, eight tRNAs are encoded on the N-strand and the other 14 genes are encoded on the J-strand (Table 2). All tRNAs have the typical
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Table 3. Summarized mitogenomic characteristics of the three buprestid species investigated in this study.

| Species     | PCGs Size (bp) | A + T (%) | AT-Skew | rRNAs Size (bp) | A + T (%) | AT-Skew | tRNAs Size (bp) | A + T (%) | AT-Skew | A + T-Rich Region Size (bp) | A + T (%) | AT-Skew |
|-------------|----------------|-----------|---------|-----------------|-----------|---------|-----------------|-----------|---------|----------------------------|-----------|---------|
| C. copipes  | 11,159         | 72.93     | −0.16   | 2004           | 77.64     | −0.03   | 1432            | 74.58     | 0.01    | 1614                       | 81.16     | −0.05   |
| C. dentata  | 11,159         | 75.23     | −0.16   | 1996           | 78.66     | −0.01   | 1429            | 76.35     | 0.02    | 1610                       | 83.60     | −0.09   |
| D. corrugata| 11,150         | 69.70     | −0.15   | 2021           | 75.41     | −0.09   | 1441            | 73.56     | 0.02    | 1668                       | 79.50     | 0.06    |
3.4. Non-Coding Region

Typically, the A + T-rich region also known as the control region (CR), is the largest non-coding region in mitogenome. The lengths of A + T-rich regions of these three species were 1614 bp (C. copipes), 1610 bp (C. dentata), 1668 bp (D. corrugata), respectively. The A + T-rich region in these three mitogenomes are located between trnl and rrnS. The A + T content (79.50–83.60%) of these three species was found to be higher than that of the whole mitogenome (71.76–76.59%), PCGs (69.70–75.23%), rRNAs (75.41–78.66%), and tRNAs (73.56–74.58%). Furthermore, compositional analysis showed that only the A + T-rich region of D. corrugata had a positive AT-skew among these three species.

In these three species, in addition to the large non-coding region, there are several small non-coding intergenic spacers, which are composed of less than 10 non-coding nucleotides in the mitochondria of most animals [75]. Nevertheless, in three species, longer than usual non-coding elements in the gene spacer region were found between the trnS2 and nad1 genes, with lengths of 18 in C. copipes, 24 in C. dentata, and 29 in D. corrugata. This spacer exists in many Coleoptera mitogenomes and may be used as a constant molecular marker of Coleoptera mitochondrial DNA [65].

3.5. Phylogenetic Analysis

A total of 18 buprestid species and two outgroups (Heterocerus parallelus and Dryops ernesti) were used for the phylogenetic relationship based on mitogenome data. In this study, phylogenetic trees utilizing three nucleotide sequence matrices (13 PCGs, 2 rRNAs, and 2 rRNAs + 13 PCGs) from 20 species were constructed using different best-fit substitution models (Table 4). The results of phylogenetic analysis based on different datasets were almost identical. Although the ML and BI trees constructed from protein genes do not have the same topology, they do not differ much in their expression results (Figures 7 and 8). Both ML and BI trees using two datasets (2 rRNAs and 2 rRNAs + 13 PCGs) produced identical topologies, with only slight differences in nodal support value (Figures 9 and 10). For the same datasets, the node support values of BI trees are always higher than ML trees, which has often occurred in many previous studies of other taxa [76–78].

Table 4. Best-fit models of three datasets used for phylogeny.

|              | ML                  | BI                  |
|--------------|---------------------|---------------------|
| 13PCGs       | GTR + F + I + G4    | GTR + F + I + G4    |
| 2rRNAs       | TVM + F + I + G4    | GTR + F + I + G4    |
| 13PCGs + 2rRNAs | GTR + F + I + G4  | GTR + F + I + G4    |
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Table 4. Best-fit models of three datasets used for phylogeny.

| Dataset          | ML             | BI             |
|------------------|----------------|----------------|
| 13 PCGs          | GTR + F + I + G4 | GTR + F + I + G4 |
| 2 rRNAs          | TVM + F + I + G4 | GTR + F + I + G4 |
| 13 PCGs + 2 rRNAs| GTR + F + I + G4 | GTR + F + I + G4 |

Figure 7. Phylogenetic relationships of 18 selected buprestid species using ML analyses based on 13 PCGs of mitogenomes. The symbols on the branches show bootstrap (ML tree). * ML bootstrap = 100; + ML bootstrap ≥ 50; − ML bootstrap < 50. Red star: means species which are newly sequenced in this study.

Figure 8. Phylogenetic relationships of 18 selected buprestid species using BI analyses based on 13 PCGs of mitogenomes. The symbols on branches show posterior probability (BI tree). * posterior probabilities = 1; + posterior probabilities ≥ 0.5; Red star: means species which are newly sequenced in this study.
Figure 8. Phylogenetic relationships of 18 selected buprestid species using BI analyses based on 13 PCGs of mitogenomes. The symbols on branches show posterior probability (BI tree). * posterior probabilities = 1; + posterior probabilities ≥ 0.5; Red star: means species which are newly sequenced in this study.

Figure 9. Phylogenetic relationships of 18 selected buprestid species inferred based on maximum-likelihood and Bayesian analyses of two rRNA genes. * ML bootstrap = 100 or posterior probabilities = 1; + ML bootstrap ≥ 50 or posterior probabilities ≥ 0.5; Red star: means species which are newly sequenced in this study.

Figure 10. Phylogenetic relationships of 18 selected buprestid species inferred based on maximum-likelihood and Bayesian analyses of 13 protein-coding genes and two rRNA genes. * ML bootstrap = 100 or posterior probabilities = 1; + ML bootstrap ≥ 50 or posterior probabilities ≥ 0.5; − ML bootstrap < 50 or posterior probabilities < 0.5. Red star: means species which are newly sequenced in this study.

The results showed that the monophyly of Agrilinae is confirmed again, as all Agrilinae species formed to a single highly supported clade. Meanwhile, two outgroup taxa obviously separated from the clade of Buprestidae. Interestingly, the target species C. copipes, C. dentata, and D. corrugata, as well as two other Buprestinae species were clustered into a
single branch with a high nodal support value. Moreover, the (C. copipes + C. dentata) + D. corrugata clade and (A. chinensis + M. acuminata) clade displayed a sister group relationship; the Chrysochroa fulgidaissima (Chrysochoinae) and Acmaeodera sp. (Polycestinae) formed a sister clade. Coomaniellini is more closely related to Dicercini than Melanophilini, which was in line with previous studies based on morphological data [79] and molecular data [15]. The genus Coomaniella, Dicerca, Anthaxia, and Melanophila were clustered together and form the Buprestinae branch based on the complete mitogenome data, however, the genus Dicerca belonged to Chrysochoinae in morphological classification. It is known that some scholars have supported the merger of Chrysochoinae and Buprestinae, indicating that there is no clear division between two subfamilies [12–15]. However, in this study, the Dicerca can be transferred from Chrysochoinae to Buprestinae based on the phylogenetic trees of this study, morphological data [79], and molecular data [15].

Although this study had contributed three new complete mitogenomes to the phylogeny of the Buprestidae, the interrelationships among the subfamilies and tribes still require more data to be determined completely. These questions will be well addressed in the future when sufficient numbers of complete mitogenomes of buprestid species are accumulated.

4. Conclusions

In this study, three mitogenomes (16,179–16,276 bp) were newly sequenced and annotated, which are the first complete mitogenome sequences to be reported in the tribes Coomaniellini and Dicercini. These three sequences have a positive AT-skew, in which, L2, I, S2, and F were the four most frequently used amino acids. Consistent with most studies on insects, only the secondary structure of trnS1 is not a clover-leaf structure, but the absence of D-arm forms a simple loop. There is a 7 bp overlapping region between atp8 and atp6, nad4, and nad4l, and the rearrangement phenomenon has not been detected in these three species. The gene cox1 (Pi = 0.159) exhibited the lowest variability. The Ka/Ks value of cox1 is the lowest, indicating that cox1 gene has a relatively low evolutionary rate. The phylogenetic results showed that Coomaniellini is more closely related to Dicercini than Melanophilini, and the monophyly of Agrilinae is confirmed again. The authors recommend that the Dicerca should be transferred from Chrysochoinae to Buprestinae. However, the samples used in this study may be too limited for a more detailed analysis. Whether Dicercini can be completely transferred from Chrysochoinae to Buprestinae needs more species, especially those of Dicercini, to be further verified. The results of this study can provide new data for phylogenetic studies of the Buprestidae and improve our understanding of the characteristics of mitogenomic and the taxonomy of Buprestidae.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes13061074/s1, Figure S1: The mitogenome maps of Coomaniella copipes (A), Coomaniella dentata (B) and Dicerca corrugata (C); Figure S2: The secondary cloverleaf structure for the tRNAs of Coomaniella copipes; Figure S3: The secondary cloverleaf structure for the tRNAs of Coomaniella dentata; Figure S4: The secondary cloverleaf structure for the tRNAs of Dicerca corrugata; Table S1: Codon usage of the protein-coding genes in Coomaniella copipes; Table S2: Codon usage of the protein-coding genes in Coomaniella dentata; Table S3: Codon usage of the protein-coding genes in Dicerca corrugata; Table S4: Summarized A+T contents of 13 PCGs for the three buprestid species investigated in this study.

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