Comparative mitogenome research revealed the phylogenetics and evolution of the superfamily Tenebrionoidea (Coleoptera: Polyphage)

Yun-Jian Hu[1], Li Hu [1], Chuan Wu[1], Tian Tian[1], Ting-Jing Li[1], and Bin Chen[1]

[1]Chongqing Normal University

March 11, 2024

Abstract

Despite the worldwide distribution and rich diversity of the superfamily Tenebrionoidea, the knowledge of the mitochondrial genomes (mtgenome) characteristics of the superfamily is still very limited and its phylogenetics and evolution remains unresolved. In present study, nineteen species of mtgenomes in Tenebrionoidea are newly sequenced and annotated, and a total of 90 mtgenomes are analyzed. There exist 37 genes for all 82 species of complete mtgenomes of 16 families investigated, and their characteristics are identical as reported mtgenomes of other Tenebrionoids. The Ka/Ks analysis suggests that all 13 PCGs have undergone a strong purifying selection. The phylogenetic analysis suggests the monophyly of Mordellidae, Ripiphoridae, Meloidae, Anthicidae, Oedemeridae, Pyrochroidae, Salpingidae, Scraptiidae, Lagriidae and Tenebrionidae, and the Mordellidae is sister to the Ripiphoridae. The "Tenebrionidae clade" and "Meloidae clade" are monophyletic, and both of them are sister groups. In the "Meloidae clade", Anthicidae is sister to Meloidae. In the "Tenebrionidae clade", the family Lagriidae and Tenebrionidae are sister groups. The divergence time analysis suggests that Tenebrionoidea originated in early Jurassic, Mordellidae, Meloidae and Oedemeridae in Cretaceous, Anthicidae, Lagriidae and Tenebrionidae in the early Cretaceous. The work lays a base for the study of mtgenome, phylogenetics and evolution of the superfamily Tenebrionoidea.

Comparative mitogenome research revealed the phylogenetics and evolution of the superfamily Tenebrionoidea (Coleoptera: Polyphage)

Yun-Jian Hu, Li Hu, Chuan Wu, Tian Tian, Ting-Jing Li and Bin Chen*

Running title: mtgenome and phylogeny of Tenebrionoidea

Chongqing Key Laboratory of Vector Insects; Institute of Entomology and Molecular Biology, College of Life Sciences, Chongqing Normal University, Chongqing 401331, China

*Correspondence: *bin.chen@cqnu.edu.cn* (BC)

Abstract . Despite the worldwide distribution and rich diversity of the superfamily Tenebrionoidea, the knowledge of the mitochondrial genomes (mtgenome) characteristics of the superfamily is still very limited and its phylogenetics and evolution remains unresolved. In present study, nineteen species of mtgenomes in Tenebrionoidea are newly sequenced and annotated, and a total of 90 mtgenomes are analyzed. There exist 37 genes for all 82 species of complete mtgenomes of 16 families investigated, and their characteristics are identical as reported mtgenomes of other Tenebrionoids. The Ka/Ks analysis suggests that all 13 PCGs have undergone a strong purifying selection. The phylogenetic analysis suggests the monophyly of Mordellidae, Ripiphoridae, Meloidae, Anthicidae, Oedemeridae, Pyrochroidae, Salpingidae, Scraptiidae, Lagriidae and Tenebrionidae, and the Mordellidae is sister to the Ripiphoridae. The "Tenebrionidae clade" and "Meloidae clade" are monophyletic, and both of them are sister groups. In the "Meloidae clade", Anthicidae is sister

to Meloidae. In the "Tenebrionidae clade", the family Lagriidae and Tenebrionidae are sister groups. The divergence time analysis suggests that Tenebrionoidea originated in early Jurassic, Mordellidae, Meloidae and Oedemeridae in Cretaceous, Anthicidae, Lagriidae and Tenebrionidae in the early Cretaceous. The work lays a base for the study of mtgenome, phylogenetics and evolution of the superfamily Tenebrionoidea.

Keywords: Coleoptera, Tenebrionoidea, mtgenome, phylogenetics, evolution

Introduction

Tenebrionoidea is a large superfamily in Coleoptera, with over 34,000 known species and 28 families (Lawrence, 1995; Ślipiński *et al* ., 2011). The species in the superfamily have the 5-5-4 tarsal formula in both sexes, with occasional 4-4-4, 3-3-3 or 3-4-4 in males. Members of the superfamily demonstrate various types of feeding strategies, the majority of which are fungivorous, xylophagous, and saprophagous. Some Tenebrionoidea species are major agricultural and forest pests, which attack commercial crops or stored products (Song *et al* ., 2018). They are widely spread throughout all terrestrial habitats from the sea shore up to dry desert and steppe habitats in all altitudinal belts, and in all types of forests, and species in arid environments are conspicuously diverse. Given these diverse feeding strategies and habitats, the morphology of the superfamily is complicated (Bouchard*et al.* , 2009). Taxonomic and phylogenetic studies are essential for understanding their biodiversity and biological characteristics, and for further protecting, utilizing and controlling them.

The framework of the classification system for Coleoptera was firstly established in 1955 and then revised in 1982, in which the Tenebrionoidea is divided into 5 lineages: 1) Tetratomidae, Melandryidae, Mordellidae, Ripiphoridae; 2) Synchroidae, Zopheridae, Prostomidae, Perimylopidae, Chalcodryidae, Tenebrionidae; 3) Oedemeridae, Stenotrachelidae, Meloidae; 4) Pythidae, Pyrochroidae, Boridae, Mycteridae, Salpingidae; and 5) Anthicidae, Aderidae, Scraptiidae (Crowson, 1955; Lawrence & Newton, 1982). In past years, a number of studies have been reported to establish the taxonomy system of Tenebrionoidea using comparative morphology data (Beutel & Friedrich, 2005; Lawrence *et al* ., 2011). Nowadays, 28 families (Mordellidae, Ripiphoridae, Anthicidae, Meloidae, Pyrochroidae, Oedemeridae, Salpingidae, Scraptiidae, Lagriidae, Tenebrionidae and so on) are widely recognized for Tenebrionoidea (Bouchard *et al* ., 2011). Despite of economic importance, the monophyletic status of many families and phylogenetic relationships in Tenebrionoidea need to be elucidated. A comprehensive phylogeny study of beetles showed the three families Mordellidae, Anthicidae and Meloidae are monophyletic, the Scraptiidae is paraphyletic based on *18S*rRNA, *16S* rRNA and *COX1* gene sequences (Hunt *et al* ., 2007). The phylogenetic analysis of Coleoptera showed that the families Tenebrionidae, Oedemeridae, Salpingidae, Mordellidae, Meloidae and Scraptiidae are monophyletic; Pyrochroidae is polyphyletic based on four gene (*16S* rRNA*, COX1, 28S and 18S*) sequences (Bocak *et al* ., 2014). The higher-level phylogenetic analysis of beetles indicated that the Tenebrionidae, Oedemeridae, Salpingidae, Pyrochroidae, Meloidae and Scraptiidae are monophyletic, whereas Anthicidae is paraphyletic based on 95 nuclear protein-coding genes from 373 beetle species (Zhang*et al.* , 2018). Almost all molecular phylogenetic analyses support the monophyly of Mordellidae and Meloidae, which is inconsistent with the phylogenetic analyses based on morphological data (Lawrence*et al* ., 2011). Due to incongruent phylogenetic relationships from different analysis, the phylogenetic relationships and monophyly among families are still not well settled, and the phylogenetic positions among some families are contradictory in different molecular studies. All of these phylogenetic issues urgently need to be elucidated.

With the characteristics of conservative gene content, simple genome organization, maternal inheritance, small genome size and higher evolution rate, complete mitochondrial genome (mtgenome) has been widely used in molecular phylogenetics, evolution, and population genetics study of insects (Cameron *et al* ., 2014). Up to date (October 2021), there have been 71 species of Tenebrionoidea complete mtgenomes to be reported in GenBank, covering 15 families in Tenebrionoidea. However, there is no complete mtgenome sequence to be reported from the families Archeocrypticidae, Stenotrachelidae and Melandryidae, and the characteristics of the mtgenomes is still little understood in the superfamily. Up to now, there have been only a few mtgenome-based phylogenetic studies at high taxa in Tenebrionoidea. A mtgenome-based phylogenetic inference of beetles indicated that the families Tenebrionidae, Oedemeridae, Anthicidae, Mordellidae and Meloidae were

monophyletic, whereas Scarptiidae was polyphyletic based on 245 mitochondrial sequences using Bayesian method (Timmermans *et al.* , 2015). The phylogenetic analysis of 37 beetle mitochondrial genes supported the Mordellidae and Meloidae to be monophyletic groups, and the Scraptiidae and Melandryidae to be paraphyletic groups using Bayesian inference (Song *et al.* , 2018). The mtgenome-based phylogeny of 51 beetles found that the monophyletic of Meloidae, Tenebrionidae, and the sister relationship of Meloidae and Tenebrionidae using Bayesian inference (Tang *et al.* , 2020). As mentioned above, previous mtgenome-based phylogenetic studies have not well resolved the phylogeny of Tenebrionoidea, and phylogenetic analysis of the superfamily need to be further evaluated.

In this study, we newly sequenced and annotated 19 species of complete mtgenomes, and comparatively analyzed the mtgenome characteristics of a total of 90 species in Tenebrionoidea. More importantly, we constructed and discussed the phylogenetic relationships at high taxa level based on these mtgenome sequences, and inferred the divergence time at main nodes in the phylogenetics in inference of fossil records. This study lays a foundation for further understanding of mtgenome characteristics, phylogenetics and evolution of the Tenebrionoidea.

Material and methods

Sample collection, sequencing and mtgenome assembly

All samples were collected in China (Table S1), and then stored in 95% alcohol at -20 °C until DNA extraction. These samples were identified in morphology, 19 species were selected for sequencing, and their identification were confirmed by subsequent *COX1* comparison with BOLD (http://boldsystems.org/index.php) (Hebert *et al* ., 2003). The total genomic DNA was extracted from the thorax and leg muscle tissue by DNeasy Blood and Tissue kit (Qiagen, Duesseldorf, Germany) according to the instructions of manufacturer. Concentration of extracted genomic DNA was determined by Qubit 2.0 (Invitrogen, Shanghai, China). The 350 bp small fragment libraries were constructed, and then sequenced using the Illumina Hiseq 2500 (San Diego, CA) with 150 bp paired-end reads in Shenzhen Huitong Biotechnology Co. Ltd (Shenzhen, China). After removing the adapters, and unpaired, short and low qualitied reads, clean reads from mtgenomes were extracted using a BLAST (Altschul *et al.* , 1990) search against known Tenebrionoidea mtgenome sequences, and then used for *de novo*mtgenome assembly with SPAdes v. 3.9.0 (Bankevich *et al.* , 2012). The contigs of mtgenome were extracted and assembled into mtgenomes through searching against the reference sequences using PRICE (paired-read iterative contig extension) by NOVOPlasty version 2.6.2 (Dierckxsens *et al.* , 2016).

Mtgenome annotation and characteristics analysis

The rough annotation of protein-coding genes (PCGs), transfer RNA genes (tRNAs), ribosomal RNA genes (rRNAs), and CR was initially identified using MITOS (http://mitos.bioinf.uni-leipzig.de/index.py) (Bernt *et al.* , 2013), and then determined in comparison of published homologous mtgenome sequences in phylogeny-close species using MEGAX (Kumar *et al.* , 2018). The tRNAs secondary structures were predicted using tRNAscan-SE Search Server v. 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe & Eddy, 1997). The annotation of the mtgenomes was corrected manually using the Geneious v. 4.8.5 (Kearse *et al.* , 2012), and final mtgenomes were submitted to the GenBank database. The secondary structures of the tRNAs were visualized and manually edited using VARNA (http://varna.lri.fr) (Darty *et al.* , 2009). The mtgenomes were visualized using the Chloroplot online server with default parameters (Zheng *et al.* , 2020). Base composition and relative synonymous codon usage (RSCU) of 90 species of mtgenome were computed with PhyloSuite desktop platform (Zhang *et al.* , 2020). AT-skew [(A - T) / (A + T)] and GC-skew [(G - C) / (G + C)] were estimated to investigate nucleotide composition bias (Perna & Kocher, 1995), and three-dimensional scatterplots of AT-Skew, GC-Skew and AT% were drawn using Origin Pro v. 9.0 (Mikrajuddin and Khairurrijal, 2009). Selection pressure of the 13 PCGs was analyzed by calculating Ka (non-synonymous mutation rates) and Ks (synonymous mutation rates) values with DnaSP v. 5.0 (Librado & Rozas, 2009), and visualized using RStudio. Sequences saturation was assessed in DAMBE v. 5.0 (Xia, 2013).

Phylogenetic analysis of Tenebrionoidea

Phylogenetic relationships of 90 species of mtgenomes (including 19 sequenced in this study) in Tenebrionoidea were deduced using three datasets and two inference methods with *Aiolocaria hexaspilota*and *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinelloidea) as outgroups. Taxonomic information for each species investigated and mtgenome accession numbers are listed in Table 1. Three datasets were concatenated using PhyloSuite platform: 1) amino acid sequence of 13 PCGs (AA); 2) nucleotide sequence at 1st and 2nd codon position of 13 PCGs (PCG12); 3) PCG12 + 2 rRNAs, respectively with excluding start codon, stop codon. Nucleotide sequences of 13 PCGs were aligned by codon-based multiple alignments using the L-INS-i algorithm and the rRNAs were aligned using the Q-INS-i strategy in MAFFT v. 7.0 (Katoh & Standley, 2013), and ambiguously aligned positions were excluded using Gblocks (Talavera & Castresana, 2007). The concatenation of aligned sequences was performed using SequenceMatrix (Vaidya *et al.* , 2011). The selection of best-fit partitioning schemes and substitution models for each dataset were calculated using PartitionFinder v. 2.0 (Lanfear *et al.* , 2016) with the settings: branch lengths as linked, model election as AICc with the greedy algorithm. Partitioning schemes and models are listed in Table S2. Two methods, maximum likelihood (ML) and Bayesian inference (BI) were employed for the deduce. ML-based phylogenetic analyses were conducted using IQ-TREE v. 1.6.8 in PhyloSuite v. 1.2.2 (Zhang *et al.* , 2020). Nodal support values were inferred with 1 000 bootstrapped replicates (BPs) (Minh *et al.* , 2013). BI analysis was conducted using MrBayes v. 3.2.6 (Ronquist *et al.* , 2012). A total of 2 000 000 generations with four chains were sampled every 1 000 generations. Posterior probabilities (PPs) were computed after discarding the first 25% of trees as the burn-in phase. The estimated sample size (ESS) $>$ 200 and the average deviation of the split frequency of less than 0.01 indicates that the runs had converged. The phylogenetic tree was visualized using FigTree v. 1.4.4 and iTOL online tool (Letunic and Bork, 2016).

Divergence time estimation

Divergence time was estimated using the uncorrelated relaxed clock model as implemented in BEAST v. 1.6.1 (Drummond and Rambaut, 2007). In order to limit the numbers of parameter for the estimation, Bayesian tree was used as a guide tree. The tree prior generated using a Yule speciation model, and all node calibrations were enforced using Normal distributions. Constraints on clade ages were enforced using three fossil calibrations. Mordellidae (*Protoripidius burmiticus*) diverged from the other lineages in the Tenebrionoidea approximately in 166.6 Mya (Cai *et al* ., 2018), Anthicidae-Meloidae (*Camelomorpha longicervix*) split at 145 Mya (Kirejtshuk *et al* ., 2008), and Tenebrionidae-Lagriidae (*Alphitopsis initialis*) split at 143.6 Mya (Kirejtshuk *et al* ., 2012). The posterior time estimation was conducted using a MCMC algorithm, and the MCMC run was sampled every 1 000 iterations until it achieved 10 000 samples, after the first 100 000 iterations were discarded as burn-in. The effective sample sizes of every node age were confirmed using Tracer v. 1.5 until every parameter being >200. The maximum clade credibility tree was calculated using TreeAnnotator v. 1.6.1, with the node times scaled to match the mean posterior estimates.

Results

Mtgenome organization

A total of 19 species of mtgenomes in Tenebrionoidea are completely sequenced in the present study (accession numbers in Table 1), and 11 species of them are reported for the first time with all in Lagriidae. All 82 species of complete mtgenomes investigated contain the typical 37 genes (including 13 PCGs, 2 rRNAs and 22 tRNAs) and one control region (CR). There are 22 genes (nine PCGs and 13 tRNAs) located on the majority coding strand (J-strand), while the other 15 genes (four PCGs, nine tRNAs and two rRNAs) are on the minority strand (N-strand; Fig. 1). These mtgenome sequences range in length from 14 777 bp (*Cerogria kikuchii*) to 16 861 bp (*Heterotarsus carinula*) with an average of 15 763 bp, and the length variation mainly results from the control region, intergenic overlap and spacers. They all display obvious AT bias with A+T content ranging from 62.7% (*Casnonidea terminata*) to 81.6% (*Pyrochroidae* sp.) and an average being 73.6%. AT-skew values range from -0.141 (*Paramarygmus* sp.) to 0.219 (*Strongylium pinfaense*), and GC-skew from -0.375 (*S. pinfaense*) to 0.366 (*Paramarygmus* sp.) (Fig. 2).

The tRNAs sizes range from 57 bp to 82 bp, and all of the tRNAs can be folded into a typical clover-

leaf structure except for*tRNA -Ser* (AGN), in which the dihydrouridine (DHU) arm is absent and a UCU anticodon is present (Fig. S1). The most frequently occurred base mismatches are U-G, U–U and A-G, and the mismatch A-G is only occurred in *tRNA-Trp* . For rRNAs, *rrnL* is located between the *trnL1* and *trnV* , ranging from 750 bp (*Anthicidae* sp.) to 1 323 bp (*Alcidodes juglans*). The length of the *rrnS* ranges from 740 bp (*Cerogira popularis*) to 1 269 bp (*Uloma* sp.), which is located between the *trnV* and CR region. The percentage of AT content in rRNAs is 68.3-84.3%. The CRs are located between the *rrnS* and*trnI* , and the percentage of AT content in this region is 72.2-96.1%.

Rearrangement events

By comparing the composition and structure of the mtgenomes of these 90 Tenebrionoidea species, a total of seven Tenebrionoidea species were found to have gene rearrangement events. These rearrangement events occurr in the three different families Lagriidae, Mordellidae and Pyrochroidae (Fig. 3). In others families, the gene order of the mtgenome is exactly the same as that of *drosophilid* . The tRNA genes have the highest frequency of rearrangement (*trnW-trnC-trnY* and *trnA-trnR-trnN-trnS-trnE-trnF* gene cluster), followed by protein coding genes. The first one is found in the*trnW-trnC-trnY* gene cluster, and shuffling of *trnW* gene and *trnC* gene occurs in three species of *Schizotus pectinicornis* (Pyrochroidae), *Pyrochroidae* sp. (Pyrochroidae) and *Anisostira rugipennis* (Lagriidae). The second one in the*A. rugipennis* of Lagriidae, *trnD* and *ATP8*transposition to the upstream of *NAD2* , which is the first rearrangement event of protein coding genes found in the family Lagriidae. The last one is found in the*trnA-trnR-trnN-trnS-trnE-trnF* gene cluster, and shuffling of*trnR* gene and *trnN* gene occurs in four species of the family Mordellidae: *Mordellidae* sp., *Mordellochroa milleri* , *Mordella atrata* and *Tomoxia bucephala* .

Codon usage of PCGs and gene selection pressure

Total PCGs nucleotide length ranges from 10 848 bp to 11 142 bp, and the AT contents ranges from 60.7% to 81.0%. Most of the PCGs initiate with the typical start codon ATN and TTG, whereas the special start codons AAC, AAT, AAA and TCA are found for *COX1* ; AAA for *COX2* ; GTG for *ND1* and *ND4L* ; AGG and AAA for *ND2* and GTG for *ND4* . The most frequently used stop codons are TAA and TAG, followed by the incomplete stop codons T and TA. The most frequently used codons are UUA (Leu2), UCU and UCA (Ser2), CGA (Arg), whereas AGC (Ser1), ACG (Thr), GCG (Ala) and CUG (Leu1) are the least used (Fig. 4). For each PCG, the Ka/Ks ratio is less than one, and the *ATP8* has the highest Ka/Ks ratio (0.33-0.67), followed by seven genes (*ND6, ND5, ND4, ND2, ND4L, ND1, ND3*) with Ka/Ks ratios of 0.17-0.42. Complex IV (*COX1, COX2* and *COX3*), Complex III (*CYTB*) and*ATP6* have low Ka/Ks ratios with range from 0.01 to 0.17 (Fig. 5). These results imply all of these 13 PCGs experienced purifying selection, especially Complex IV and Complex III.

Phylogenetic relationships

Substitution saturation tests show no saturation for three datasets AA, PCG12 and PCG12 + rRNAs (Iss $<$ Iss.cSym or Iss.cAsym, $p<$ 0.05) (Table 2), which proposes that these three datasets be appropriate for phylogenetic construction based on ML and BI. Six trees generated using these three datasets and both ML and BI are slightly different in topology (Figs. 6-7; Figs. S3-S6). The Ciidae is located at base of all phylogenetic trees, followed by families Mordellidae + Ripiphoridae. The Mordellidae + Ripiphoridae, Mordellidae and Ripiphoridae all looks monophyletic (PP = 1; BP = 100) and the later two appear sister groups each other. All remaining families also appear monophyletic, and the family Aderidae seems sister with "Meloidae clade" + "Tenebrionidae clade". Both "Tenebrionidae clade" and "Meloidae clade" looks monophyletic with both of them being sister groups (Fig. 6). In the "Meloidae clade", the families Meloidae + Anthicidae look a monophyletic (PP = 1; BP = 100), and it appears sister to the "Oedemeridae clade", and the Meloidae and Anthicidae look monophyletic (PP = 1; BP = 100), and a sister each other. In the "Oedemeridae clade", the Oedemeridae, Pyrochroidae, Salpingidae and Scraptiidae seem monophyletic (PP = 1; BP = 100), and Salpingidae looks a sister with Scraptiidae. In the "Tenebrionidae clade", the family Lagriidae and Tenebrionidae are monophyletic (PP = 1; BP = 90-100), and both of them are sister groups each other. In Lagriidae, the subfamily Adeliinae is based at the subfamilies Lagriinae and Statininae, both of which look monophyletic

(PP = 1; BP = 100) and are sister groups each other. In Tenebrionidae, the subfamily Pimeliinae appears monophyletic group (PP = 1; BP = 100) and is located at the base of Tenebrionidae. The Alleculinae and Stenochiinae look monophyletic (PP = 1; BP = 100), and the subfamilies Tenebrioninae and Diaperinae appears polyphyletic groups.

Six trees generated using AA, PCG12 and PCG12 + 2 rRNAs datasets and both ML and BI are slightly different in topology. For AA dataset, the topologies using ML and BI are different in the positions of Prostomidae. Prostomidae and Tetratomidae are clustered as one clade in ML tree, but not in BI tree. For PCG12 dataset, two same topologies of trees from BI and ML differ from two topologies of AA dataset in phylogenetic relationship of the "Oedemeridae clade". For PCG12 + 2 rRNAs dataset, the positions of major families are the same as the four topologies of AA and PCG12 datasets, with only a few differences in the "Oedemeridae clade".

Divergence time

The AA dataset was used to estimate divergence time because AA had higher node support values than others in the initial phylogenetic assessment using Bayesian appraoch. Based on three fossil calibrations points of *Protoripidius burmiticus* (166.6 Mya),*Camelomorpha longicervix* (145 Mya) and *Alphitopsis initialis* (143.6 Mya) (filled red cycles in Fig. 8), the superfamily Tenebrionoidea was inferred to originate in the early Jurassic (192.6 Mya, 95% confidence interval (CI): 179.3-208.7 Mya), with most families subsequently diverging in the Jurassic and early Cretaceous (Fig. 8). The family Mordellidae and Ripiphoridae is among the earliest diverged families in the superfamily, and is estimated to originate at 115.7 and 126.6 Mya in the Cretaceous, respectively. In the "Meloidae clade", the family Meloidae is estimated to be derived at 105 Mya in the Cretaceous, the Anthicidae at 123.8 Mya in the early Cretaceous, and the Oedemeridae at 100.9 Mya in the middle Cretaceous. In the "Tenebrionidae clade", the Lagriidae is estimated to originate at 134.3 Mya in the early Cretaceous, and the Tenebrionidae at 128.9 Mya in the early Cretaceous. In the family Lagriidae, the subfamily Statiriinae diverged 97.6 Mya in the late Cretaceous, and the Lagriinae diverged 78.6 Mya in the late Cretaceous. In the family Tenebrionidae, the subfamilies Pimeliinae, Alleculinae and Stenochiinae originated at 71.1, 53.6 and 53.3 Mya in the Paleogene, respectively. All of these families/subfamilies are proposed to be monophyletic and confirmed in relationships in the phylogenetic analyses using different data or inferring methods, and others are not determined for their monophyly or relationships or have a few species included in the phylogenetic analyses, and therefore they are not given an inferring of divergence time.

Discussion

Characteristics of Tenebrionoidea mtgenomes

These 90 mtgenomes investigated in the present study in the superfamily Tenebrionoidea have a length variation from 14 777 bp to 16 861 bp, and the length variation mainly stems from CR, intergenic overlap and spacers, which is consistent with earlier reports in Tenebrionoids (Burger *et al.* , 2003). The nucleotide composition for all species exhibits obvious AT bias with high A+T content, similar as earlier reports in Tenebrionoids (Jie *et al.* , 2016). All tRNA genes can form a complete clover secondary structure, except for*tRNA -Ser* (AGN) that lacks the DHU arm, which seem to be a common feature of Tenebrionoidea (Zhang *et al.* , 2016; Song*et al.* , 2018). There are some rearrangement events in some species of the family Mordellidae, Lagriidae and Pyrochroidae. The shuffling of the *trnR* and *trnN* genes (*trnA-trnR-trnN-trnS-trnE-trnF* gene cluster) is found in all specials in the family Mordellidae; the translocation of the*trnD* and *ATP8* genes is found in the *A. rugipennis*of Lagriidae for the first time; the shuffling of the *trnC* and*trnW* genes (*trnW-trnC-trnY* gene cluster) is found in Pyrochroidae and Lagriidae. These gene rearrangement may be produced by abnormal priming of mitochondrial replication by a tRNA molecule or tandem duplications, which can provide an important reference for Tenebrionoidea phylogeny inference (Boore & Brown, 1998; Boore *et al.* , 1998; Timmermans & Vogler, 2012; Cameron, 2014). The ATN and TTG are mainly used as the start codon, and TAA and TAG as the stop codon for the 13 PCGs, which is similar as other mtgenome sequences in Tenebrionoidea (Du *et al.* , 2017). The Ka/Ks ratio is lower than one for all PCGs, which is consistent with

earlier studies in Tenebrionoidea. The *COX1* gene has experienced strong evolutionary pressure in order to maintain its own functional requirements, whereas *ATP8* has experienced weak evolutionary pressures with allowing more mutations to accumulate in the mtgenome (Ou *et al.* , 2016; Bai *et al.* , 2018).

Overview of phylogenetic relationships

A total of 16 families are included in the phylogenetics and evolution analysis in the Tenebrionoidea, in which there are 10 families with at least two representative species included. The family Ciidae seems to be earliest derived in these families, followed Mordellidae + Ripiphoridae, and Aderidae + "Meloidae clade" + "Tenebrionidae clade". Ciidae was historily placed in the Cucujoidea (Crowson, 1955) and then to the superfamily Tenebrionoidea mainly based on characteristics of the aedeagus and the larval abdomen (Crowson, 1960). It was proposed to be a monophyly based on 18S and *COX1* genes using ML and BI methods, and demonstrated to be a either sister to Nitidulidae based on the reduced sample or at the base of the cucujoid-tenebrionoid assemblage based on the entire sample (Buder *et al.* , 2008). It was considered basal tenebrionoids based on 516 adult and larval morphological characteristics from 359 beetle taxa (Lawrence *et al* ., 2011). The present study also suggests the family to be the basal tenebrionoids, but further investigation is necessary to elucidate its place with the inclusion of more species.

Mordellidae + Ripiphoridae, Mordellidae and Ripiphoridae are all proposed to be monophyletic, and the two families demonstrate to be sister groups each other in the present study. The Mordellidae + Ripiphoridae was also proposed monophyletic in earlier molecular phylogeny inference based on five nuclear and mitochondrial genes with 300 genera in Tenebrionoidea using ML (Gunter *et al.* , 2014). The Mordellidae was also proposed to be monophyletic in the study based on four molecular genes (*18S*rRNA, *28S* rRNA, *rrnL* and *COX1*) with 128 species in Tenebrionoidea using ML (Batelka *et al.* , 2016). The Ripiphoridae was proposed to be monophyletic from a molecular phylogenetic analysis based on eight nuclear genes with 367 species in Tenebrionoidea using Bayesian method (Mckenna *et al.* , 2015). However, it was proposed to be paraphyletic in the molecular phylogenetic study based on four mitochondrial and four nuclear gene fragments across 404 taxa (including 250 tenebrionid species) using ML (Kergoat *et al.* , 2014a), which suggests that the monophyletic status of the Ripiphoridae remains uncertain. The two families were not proposed to be sister group in the earlier molecular phylogenetic study (Gunter *et al.* , 2014; Kergoat *et al.* , 2014a), which due to the monophyletic status of Ripiphoridae remains uncertain.

Aderidae was proposed to be a monophyletic lineage in the earlier molecular phylogenetic study (Gunter *et al.* , 2014). There is only species in Aderidae to be included in the present study, which be formed a monophyly with "Meloidae clade" + "Tenebrionidae clade", and its position and monophyletic status are yet to be resolved with more species to involved. The "Meloidae clade" + "Tenebrionidae clade", "Meloidae clade" and "Tenebrionidae clade" are all proposed to be monophyletic, which are consistent with earlier studies based on five nuclear and mitochondrial genes with 300 genera in Tenebrionoidea using ML (Gunter *et al.* , 2014) and eight mitochondrial and nuclear gene with 404 taxa in Coleoptera using ML (Kergoat *et al.* , 2014a).

Phylogenetic relationships of "Meloidae clade"

The Meloidae + Anthicidae, Meloidae and Anthicidae are all proposed to be monophyletic, and the two families demonstrate to be sister groups each other in the present study. These results are consistent with earlier studies. The monophyly of Meloidae + Anthicidae was proposed based on 245 mitochondrial sequences in Coleoptera, including 159 newly sequenced full or partial mtgenomes using PhyloBayes (Timmermans*et al.* , 2015). The monophyly of Meloidae was proposed based on 4 818 nuclear genes in 146 species in beetles using ML (Mckenna *et al.* , 2019). The monophyly of Anthicidae was proposed based on*18S* rRNA, *16S* rRNA and *COX1* gene sequences from 340-taxa using BI (Hunt *et al* ., 2007), and also based on other molecular phylogenetic studies (Kergoat *et al.* , 2014a; Timmermans*et al.* , 2015; Mckenna *et al.* , 2019). The sister relationship of Anthicidae and Meloidae was proposed based on the morphology characteristics of mesothoracic glands (Hemp and Dettner, 1997), and also based on mitochondrial and nuclear genes (Timmermans*et al.* , 2015; Mckenna *et al.* , 2019).

In the "Oedemeridae clade", the family Prostomidae seems to be located at the base of "Oedemeridae clade", followed Oedemeridae and Trictenotomidae + Tetratomidae. Prostomidae was proposed a sister to the "pythid-pyrochroid-lineage" (including Trictenomatidae, Pyrochroidae, Salpingidae and so on) based on morphology characteristics of the maxillary articulatory area, the abdominal tergite IX extending to the ventral side of the segment, and the strongly pronounced prognathous condition (Schunger *et al.* , 2003). However, Prostomidae and Tetratomidae are clustered as one clade in ML tree, and the phylogenetic position of Prostomidae remained unresolved in the present analyses.

The family Oedemeridae is proposed to be monophyletic, which is consistent with the earlier phylogenetic study based on four gene (*16S* rRNA*, COX1, 28S* and *18S* rRNA) sequences from 8441 taxa of Coleoptera, which removed misplaced single specimens and minor clades (Bocak *et al.* , 2014). However, it was proposed to be paraphyletic in the phylogenetic study based on mitochondrial and nuclear genes (Gunter *et al.* , 2014; Zhang *et al.* , 2018), which suggests that the monophyletic status of the Oedemeridae need be further determined with more species included. Trictenotomidae and Tetratomidae are clustered as one clade using BI in this study, whereas Trictenotomidae was a sister to Boridae in earlier phylogenetic studies based on nuclear genes (Mckenna*et al.* , 2019). There is only species in Trictenotomidae and Tetratomidae to be included in the present study, which suggests that the monophyletic status of the Trictenotomidae and Tetratomidae remains uncertain, and its position and monophyletic status are yet to be resolved with more species to involved.

Zopheridae and Pyrochroidae are clustered as one clade, and Pyrochroidae seems monophyletic in the present study. The monophyly of Pyrochroidae was also proposed in the earlier phylogenetic study based on 95 nuclear protein-coding genes in 373 beetle species using ML and BI (Zhang *et al.* , 2018), and in other phylogenetic studies based on mitochondrial and nuclear genes (Gunter *et al.* , 2014; Kergoat *et al.* , 2014a; Mckenna*et al.* , 2019). Zopheridae was a sister to Tetratomidae in earlier phylogenetic studies based on mitochondrial and nuclear genes (Kergoat*et al.* , 2014a), which suggests that the position and monophyletic status of Zopheridae and Pyrochroidae need be further determined. The families Salpingidae and Scraptiidae seem monophyletic, and are sister groups each other in the present study. The monophyly of Salpingidae and Scraptiidae was also proposed in the earlier phylogenetic studies based on mitochondrial and nuclear genes (Bocak *et al.* , 2014; Zhang*et al.* , 2018; Mckenna *et al.* , 2019), whereas Scraptiidae was proposed a paraphyletic group in other molecular phylogenetic studies (Hunt *et al* ., 2007; Kergoat *et al.* , 2014a; Mckenna*et al.* , 2015). The sister relationship of Salpingidae and Scraptiidae remains unclear due to the limited inclusion of only two or three species. Therefore, the position and monophyletic status of Salpingidae and Scraptiidae need be further determined with more species included.

Phylogenetic relationships of "Tenebrionidae clade"

In the "Tenebrionidae clade", the family Lagriidae and Tenebrionidae are monophyletic, and both of them are sister groups each other in the present study. The classification of Lagriidae has been debated over the years, and some scholars argue that Lagriidae should be classified as a subfamily within Tenebrionidae. However, the beetles of Lagriidae are leafivorous like beetles of Chrysomelidae, and morphological characteristics of Lagriidae adapt much more for free-moving and leaf-feeding than the beetles from other subfamilies in Tenebrionidae. The monophyly of Lagriidae was proposed in the earlier morphology study based on the characteristics of abdominal defense glands, female reproductive tract, mouthparts morphology and structure of the wings in Lagriid and Tenebrionoid (Doyen & Tschinkel, 1982). The monophyly was also proposed in earlier phylogenetic inference based on mtgenomes and nuclear genes (Gunter *et al.* , 2014; Kergoat *et al.* , 2014a). The mtgenome-based phylogeny of 36 species in Tenebrionidae suggested the monophyly of Lagriidae and Tenebrionidae, and their sister relationships based on PCG123 datasets using BI and ML (Wu *et al.* , 2021), which is consistent with the present study. The monophyly of Tenebrionidae was also proposed in some earlier phylogenetic studies based on mitochondrial and nuclear genes (Hunt *et al* ., 2007; Timmermans *et al.* , 2015; Mckenna*et al.* , 2019).

The present study suggests the phylogenetic relationships of Adeliinae + (Lagriinae + Statininae) in the family Lagriidae, and the monophyly of Lagriinae and Statininae. The monophyly of Lagriinae was proposed

in earlier phylogenetic studies based on the morphology characteristics (Doyen, 1989), and also based on mitochondrial and nuclear genes (Gunter*et al* ., 2014; Wu *et al* ., 2021). The present study supports the monophyly of the subfamily Statiriinae for the first time. The Adeliinae is at the base of the family Lagriidae in this study, which is consistent with the earlier mtgenome-based study (Wu *et al* ., 2021). The monophyly of Adeliinae in Lagriidae is not yet determined due to only one species to be included.

In the family Tenebrionidae, the present study supports the monophyly of the subfamily Pimeliinae, Stenochiinae and Alleculinae, whereas Tenebrioninae and Diaperinae are recognized as a polyphyly. The subfamily Pimeliinae was also proposed to be monophyletic in the earlier studies based on nuclear genes and mitochondrial genes (Gunter *et al.* , 2014; Wu *et al* ., 2021). The present study supports the monophyly of the subfamilies Stenochiinae and Alleculinae in Tenebrionidae, which was also proposed in earlier phylogenetic studies based on mitochondrial and nuclear genes (Kergoat *et al.* , 2014a; Wu *et al* ., 2021). The subfamily Tenebrioninae and Diaperinae in these molecular-based studies are found to be polyphyletic, which is need to be elucidated with more species involved.

Evolution of Tenebrionoidea

The Tenebrionoidea is inferred to origin in the early Jurassic (179.3-208.7 Mya) based on the mtgenomes and fossil calibrations points in the present study, which is consistent with earlier evolution studies in Coleoptera based on mitochondrial and nuclear genes (Mckenna *et al.* , 2015; Zhang *et al.* , 2016; Cai *et al.* , 2022). The present results suggest that most families subsequently diverged in the Cretaceous. Angiosperms replaced the previously dominant gymnosperms during the Cretaceous, and the warm and humid environment had been produced in Cretaceous, which provided food and habitat for the families in Tenebrionoidea. The family Ciidae seems to be earliest derived in these families in the present study, which is inconsistent with earlier evolution studies (Kergoat *et al.* , 2014b). The divergence time of Ciidae is not yet determined due to only one species to be included. The family Mordellidae and Ripiphoridae is among the earliest diverged families in the superfamily, Mordellidae and Ripiphoridae are estimated to originate at 115.7 and 126.6 Mya in the Cretaceous, which is consistent with the previous evolution study result based on 95 nuclear protein-coding genes in 373 beetle species using ML and BI (Zhang *et al.* , 2018). In the "Meloidae clade", the families Meloidae (105 Mya), Anthicidae (123.8 Mya) and Oedemeridae (100.9 Mya) originated in the Cretaceous, which is consistent with earlier evolution studies based on mitochondrial and nuclear genes (Misof *et al.* , 2014; Kergoat*et al.* , 2014b). In the "Tenebrionidae clade", the family Lagriidae (134.3 Mya) is proposed to be derived in the early Cretaceous, which is similar with the evolution study based on mitochondrial and nuclear genes in 404 beetle species (Kergoat *et al.* , 2014b). The family Tenebrionidae (128.9 Mya) is suggested to be derived in the early Cretaceous, which is consistent with the results of the previous evolution study based on 4 818 nuclear genes (Mckenna *et al.* , 2019).

In the family Lagriidae, the subfamilies Lagriinae (78.6 Mya) and Statiriinae (97.6 Mya) are proposed to be derived in the late Cretaceous for the first time. In the family Tenebrionidae, the present study suggests the origin of the subfamilies Alleculinae (53.6 Mya) and Stenochiinae (53.3 Mya) in the Paleogene, but is inconsistent with the results of the earlier evolution study (Kergoat *et al.* , 2014b), which be due to differences in the taxa included, fossils constraints and analysis methods applied. In further research, more accurate estimates of divergence times are necessary with more precise fossil records for calibration and more complete sampling.

Conclusion

This is the first comprehensive study on the mtgenomes characteristics and mtgenome-based phylogenetics in Tenebrionoidea. A total of 19 species of mtgenomes in Tenebrionoidea are newly sequenced and annotated. The comprehensive analysis of 90 mtgenome sequences in Tenebrionoidea suggests that the AT-skew, length variation, and codon usage are consistent with other reported mtgenomes in Tenebrionoidea. The families Mordellidae, Meloidae, Anthicidae, Oedemeridae, Pyrochroidae, Salpingidae, Scraptiidae, Lagriidae and Tenebrionidae are suggested to be monophyletic. Ciidae is at the base of the superfamily of Tenebrionoidea, and the Mordellidae is sister to the Ripiphoridae. The "Tenebrionidae clade" and "Meloidae clade" are mono-

phyletic, and both of them are sister groups. In the "Meloidae clade", Anthicidae is sister to Meloidae. In the "Tenebrionidae clade", the family Lagriidae and Tenebrionidae are sister groups. In Lagriidae, the subfamily Adeliinae is based at the subfamilies Lagriinae + Statininae. In Tenebrionidae, the subfamily Pimeliinae, Alleculinae and Stenochiinae look monophyletic, Tenebrioninae and Diaperinae are polyphyletic. The divergence time analysis suggests that Tenebrionoidea originated in early Jurassic, Mordellidae, Meloidae and Oedemeridae in Cretaceous, Anthicidae, Lagriidae and Tenebrionidae in the early Cretaceous.

Availability of data and materials

All data are available as tables and figures in the main paper and its supplementary files. The GenBank accession numbers for the 19 mtgenomes generated in the present study with detailed information in Table S1.

Competing interests

The authors declare that they have no competing interests.**Abbreviations**

Mtgenome: mitochondrial genome; PCGs: protein-coding genes; rRNAs: ribosomal RNA genes; tRNAs: transfer RNA genes; CR: control region; RSCU: relative synonymous codon usage; BI: Bayesian inference; ML: Maximum likelihood.

Ethics approval and consent to participate

Not applicable.

Funding

This research was supported by the following, The National Natural Science Foundation of China (31872262, 31672363), National Key Program of Science and Technology Foundation Work of China (2015FY210300).

Author Contributions

Conceived and designed the research: BC, YJH, LH, TJL. Performed the specimens collecting and experiments: BC, CW, TT. Analyzed the data and wrote the paper: YJH, LH, BC, TJL.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* ,**215** , 403–410.

Bai, Y., Li, C., Yang, M. & Liang, S. (2018) Complete mitochondrial genome of the dark mealworm Tenebrio obscurus Fabricius (Insecta: Coleoptera: Tenebrionidae). *Mitochondrial DNA Part B-Resource* ,**3** , 171–172.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A. & Pevzner, P.A. (2012) SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* , **19** , 455-77.

Batelka, J., Kundrata, R. & Bocak, L. (2016) Position and Relationships of Ripiphoridae (Coleoptera: Tenebrionoidea) Inferred from Ribosomal and Mitochondrial Molecular Markers. *Annales Zoologici* ,**66** (1), 113-123.

Bernt, M., Donath, A., Juhling, F., Externbrink, F., Florentz, C., Fritzsch, G., Putz, J., Middendorf, M. & Stadler, P.F. (2013) MITOS: Improved de novo metazoan mitochondrial genome annotation.*Molecular Phylogenetics and Evolution* , **69** , 313-9.

Bernt, M., Merkle, D., Ramsch, K., Fritzsch, G., Perseke, M., Bernhard, D., Schlegel, M., Stadler, P.F. & Middendorf, M. (2007) CREx: inferring genomic rearrangements based on common intervals. *Bioinformatics* ,**23** , 2957-8.

Beutel, R.G & Friedrich, F. (2005) Comparative study of larvae of Tenebrionoidea (Coleoptera: Cucujiformia). *European Journal of Entomology* , **102** , 241–264.

Bocak, L., Barton, C., Crampton-Platt, A., Chesters, D., Ahrens, D. & Vogler, A.P. (2014) Building the Coleoptera tree-of-life for >8000 species: composition of public DNA data and fit with Linnaean classification. *Systematic Entomology* , **39** , 97-110.

Boore, J. L., & Brown, W. M. (1998) Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. *Current opinion in genetics & development* , **8** (6), 668-674.

Boore, J. L., Lavrov, D. V., & Brown, W. M. (1998) Gene translocation links insects and crustaceans. *Nature* , **392** (6677), 667-668.

Bouchard, P., Bousquet, Y., Davies, A., Alonso-Zarazaga, M., Lawrence, J., Lyal, C., Newton, A., Reid, C., Schmitt, M., Slipinski, A. & Smith, A. (2011) Family-Group Names in Coleoptera (Insecta). *ZooKeys* ,**88** , 1-972.

Bouchard, P., Grebennikov, V.V., Smith, A.B.T. & Douglas, H. (2009) Biodiversity of Coleoptera. In: Insect Biodiversity: Science and Society (Eds. Foottit, R.G. & Adler, P.H.), Blackwell Publishing, Oxford, 265–301.

Buder, G., Grossmann, C., Hundsdoerfer, A. & Klass, K.D. (2008) A Contribution to the Phylogeny of the Ciidae and its Relationships with Other Cucujoid and Tenebrionoid Beetles (Coleoptera: Cucujiformia).*Arthropod Systematics and Phylogeny* , **66** (2), 165-190.

Burger, G., Gray, M.W. & Lang, B. F. (2003) Mitochondrial genomes: anything goes, *Trends Genet* , **19** , 709–716.

Cai, C., Tihelka, E., Giacomelli, M., Lawrence, J. F., Ślipiński, A., Kundrata, R., Yamamoto, S., Thayer, M. K., Newton, A. F., Leschen, R. A., Gimmel, M. L., Lü, L., Engel, M. S., Huang, D., Pisani, D. & Donoghue, P. C. (2022) Integrated phylogenomics and fossil data illuminate the evolution of beetles. *Royal Society Open Science* ,**9** (3), 211771.

Cai, C., Yin, Z. & Huang, D. (2018) A new ripiphorid beetle from Upper Cretaceous Burmese amber sheds light on early evolution of the extant subfamily Ripidiinae (Coleoptera: Ripiphoridae). *Comptes Rendus Palevol* , **17** (6), 351-356.

Cameron, S.L. (2014) Insect Mitochondrial Genomics: Implications for Evolution and Phylogeny. *Annual Review of Entomology* ,**59** , 95-117.

Crowson, R.A. (1955) *The Natural Classification of the Families of Coleoptera.* Nathaniel Lloyd, London.

Crowson, R.A. (1960) The phylogeny of Coleoptera. *Annual Review of Entomology* , **5** , 111-134.

Darty, K., Denise, A. & Ponty, Y. (2009) VARNA: Interactive drawing and editing of the RNA secondary structure. *Bioinformatics* ,**25** , 1974-5.

Dierckxsens, N., Mardulyn, P & Smits, G. (2016) NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Research* , **45** (4), e18.

Dias, G., Lino-Neto, J., Mercati, D., Fanciulli, P. P., Lupetti, P., & Dallai, R. (2022) The sperm ultrastructure of members of basal Tenebrionoidea (Coleoptera). *Arthropod Structure & Development* ,**66** , 101129.

Doyen, J. T. (1989) Reconstitution of Coelometopini, Tenebrionini and related tribes of America north of Colombia (Coleoptera: Tenebrionidae).*Journal of the New York Entomological Society* , 277-304.

Doyen, J. T. & Tschinkel, W. R. (1982) Phenetic and cladistic relationships among tenebionid beetles (Coleoptera). *Systematic Entomology* , **7** , 127–183.

Drummond, A. J., & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC evolutionary biology* ,**7** (1), 1-8.

Du, C., Zhang, L., Lu, T., Ma, J., Zeng, C., Yue, B., & Zhang, X. (2017) Mitochondrial genomes of blister beetles (Coleoptera, Meloidae) and two large intergenic spacers in *Hycleus* genera. *Bmc Genomics* , **18** (1), 1-15.

Falin, Z. H. 2003. Phylogenetic analysis and revision of the genera and subfamilies of the Ripiphoridae (Coleoptera). PhD Thesis, University of Kansas, 560 pp. University Microfilms International, Ann Arbor, Michigan.

Gunter, N.L., Levkaničová, Z., Weir, T.H., Ślipiński, A., Cameron, S.L. & Bocak, L. (2014) Towards a phylogeny of the Tenebrionoidea (Coleoptera). *Molecular Phylogenetics and Evolution* , **79** , 305-312.

Hebert, P.D., Cywinska, A., Ball, S.L. & Dewaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of The Royal Society B-Biological Sciences* , **270** , 313-321.

Hemp, C., & Dettner, K. (1997) Morphology and chemistry of mesothoracic glands in anthicid beetles (Coleoptera: Anthicidae). *Entomologia generalis* , **22** (2), 97-108.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O.S., Wild, R., Hammond, P.M., Ahrens, D., Balke, M., Caterino, M.S., Gomez-Zurita, J., Ribera, I., Barraclough, T.G., Bocakova, M., Bocak, L. & Vogler, A.P. (2007) A Comprehensive Phylogeny of Beetles Reveals the Evolutionary Origins of a Superradiation. *Science* , **318** , 1913-6.

Jie, H., Lei, M., Li, P., Feng, X., Zeng, D., Zhao, G., Zhu, J., Zhang, C., Yu, M., Huang, Y. & Chen, Q. (2016) The complete nucleotide sequence of the mitochondrial genome of *Epicauta aptera* Kaszab.*Mitochondrial DNA Part B* , **1** (1), 489-490.

Katoh, K. & Standley, D.M. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability.*Molecular Biology and Evolution* , **30** , 772-80.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* , **28** , 1647-9.

Kergoat, G.J., Bouchard, P., Clamens, AL., Abbate, J.L., Jourdan, H., Jabbour-Zahab, R., Genson, G., Soldati, L. & Condamine, F.L. (2014b) Cretaceous environmental changes led to high extinction rates in a hyperdiverse beetle family. *BMC Evolutionary Biology* ,**14** , 220.

Kergoat, G.J., Soldati, L., Clamens, A. L., Jourdan, H., Jabbour-Zahab, R., Genson, G., Bouchard, P. & Condamine, F.L. (2014a) Higher level molecular phylogeny of darkling beetles (Coleoptera: Tenebrionidae).*Systematic Entomology* , **39** , 486-99.

Kirejtshuk, A. G., & Azar, D. (2008) New taxa of beetles (Insecta, Coleoptera) from Lebanese amber with evolutionary and systematic comments. *Alavesia* , **2** (15), e46.

Kirejtshuk, A. G., Nabozhenko, M. V., & Nel, A. (2012) First mesozoic representative of the subfamily tenebrioninae (Coleoptera, Tenebrionidae) from the lower cretaceous of Yixian (China, Liaoning).*Entomological review* , **92** (1), 97-100.

Kumar, S., Stecher, G., Li, M., Knyaz, C & Tamura, K. (2018) MEGA-X: molecular evolutionary genetics analysis across computing platforms.*Molecular Biology and Evolution* , **35** (6), 1547-1549.

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T. & Calcott, B. (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution formolecular and morphological phylogenetic analyses.*Molecular biology and evolution* , **34** (3), 772-773.

Lawrence, J.F. (1995) Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names).*Biology, Phylogeny, and Classification of Coleoptera* , **2** , 779-1006.

Lawrence, J. F., & Newton Jr, A. F. (1982) Evolution and classification of beetles. *Annual review of ecology and systematics,***13** (1), 261-290.

Lawrence, J.F., Ślipiński, A., Seago, A.E., Thayer, M.K., Newton, A.F. & Marvaldi, A.E. (2011) Phylogeny of the Coleoptera Based on Morphological Characters of Adults and Larvae. *Annales Zoologici* ,**61** , 1-217.

Letunic, L. & Bork, P. (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research* , **44** (W1), W242–245.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* ,**25** (11), 1451-1452.

Lowe, T.M. & Eddy, S.R. (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research* , **25** , 955-64.

McKenna, D.D., Shin, S., Ahrens, D., Balke, M., Beza-Beza, C., Clarke, D.J., Donath, A., Escalona, H.E., Friedrich, F., Letsch, H., Liu, S., Maddison, D., Mayer, C., Misof, B., Murin, P.J., Niehuis, O., Peters, R.S., Podsiadlowski, L., Pohl, H., Scully, E.D., Yan, E.V., Zhou, X., Ślipiński, A. & Beutel, R.G. (2019) The evolution and genomic basis of beetle diversity. *Proceedings of the National Academy of Sciences* , **116** , 24729-37.

McKenna, D.D., Wild, A.L., Kanda, K., Bellamy, C.L., Beutel, R.G., Caterino, M.S., Farnum, C.W., Hawks, D.C., Ivie, M.A., Jameson, M.L., Leschen, R.A.B., Marvaldi, A.E., McHugh, J.V., Newton, A.F., Robertson, J.A., Thayer, M.K., Whiting, M.F., Lawrence, J.F., Ślipiński, A., Maddison, D.R. & Farrell, B.D. (2015) The beetle tree of life reveals that Coleoptera survived end-Permian mass extinction to diversify during the Cretaceous terrestrial revolution. *Systematic Entomology* ,**40** , 835-80.

Mikrajuddin, A. & Khairurrijal, A. (2009) simple method for determining surface porosity based on SEM images using Origin Pro software.*Indonesian Journal of Physics* , **20** , 37–41.

Minh, B.Q., Nguyen, M.A.T. & von Haeseler, A. (2013) Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution* , **30** , 1188-95.

Misof, B., Liu, S., Meusemann, K., Peters, R. S., Donath, A., Mayer, C., ... & Zhou, X. (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science* , **346** (6210), 763-767.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution* ,**32** , 268-74.

Ou, J., Yao, F. J., Li, Y., Yang, X.Y., Jin, C. & Wei, Z.M. (2016) The complete mitochondrial genome of the confused flour beetle Tribolium confusum (Coleoptera: Tenebrionidae). *Mitochondrial DNA* ,**27** (2), 958-959.

Perna, N.T. & Kocher, T.D. (1995) Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *Journal of Molecular Evolution* , **41** , 353-358.

Ponomarenko, A. G., & Prokin, A. A. (2015) Review of paleontological data on the evolution of aquatic beetles (Coleoptera).*Paleontological Journal* , **49** (13), 1383-1413.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* ,**61** , 539-42.

Schunger, I., Beutel, R.G. & Britz, R. (2003) Morphology of immature stages of Prostomis mandibularis (Coleoptera: Tenebrionoidea: Prostomidae). *Eur. J. Entomol.* , **100** (3), 357-370.

Sheffield, N.C., Song, H., Cameron, S.L. & Whiting, M.F. (2008) A comparative analysis of mitochondrial genomes in Coleoptera (Arthropoda: Insecta) and genome descriptions of six new beetles. *Molecular Biology and Evolution* , **25** , 2499-509.

Ślipiński, S.A., Leschen, R.A.B. & Lawrence, J.F. (2011) Order Coleoptera Linnaeus, 1758. Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness (ed. by Zhang Z. Q.). *Zootaxa* , **3148** , 203– 208.

Song, N., Liu, H.Y., Yang, X.J., Zhao, X.C. & Lin, A.L. (2018) Complete mitochondrial genome of the darkling beetle Gonocephalum outreyi (Coleoptera: Tenebrionidae) with phylogenetic implications.*Journal of Asia-Pacific Entomology* , **21** , 721-30.

Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* , **56** , 564-577.

Tang, P.A., Feng, R.Q., Zhang, L., Wang, J., Wang, X.T., Zhang, L.J. & Yuan, M.L. (2020) Mitochondrial genomes of three Bostrichiformia species and phylogenetic analysis of Polyphaga (Insecta, Coleoptera).*Genomics* , **112** , 2970-7.

Timmermans, M.J.T.N., Barton, C., Haran, J., Ahrens, D., Culverwell, C.L., Ollikainen, A., Dodsworth, S., Foster, P.G., Bocak, L. & Vogler, A.P. (2015) Family-Level Sampling of Mitochondrial Genomes in Coleoptera: Compositional Heterogeneity and Phylogenetics. *Genome Biology and Evolution* , **8** , 161-75.

Timmermans, M. J. & Vogler, A. P. (2012) Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea). *Molecular Phylogenetics and Evolution* , **63** (2), 299-304.

Vaidya, G., Lohman, D.J. & Meier, R. (2011) SequenceMatrix: concatenation software for the fast assembly of multi–gene datasets with character set and codon information. *Cladistics* ,**27** (2), 171–180.

Watt, J.C. (1974) A revised subfamily classification of Tenebrionidae (Coleoptera). *New Zealand Journal of Zoology* , **1** , 381-452.

Wu, C., Zhou, Y., Tian, T., Li, T. J. & Chen, B. (2021) First report of complete mitochondrial genome in the subfamily Alleculinae and mitochondrial genome-based phylogenetics in Tenebrionidae (Coleoptera: Tenebrionoidea). *Insect Science* , **29** (4), 1226-1238.

Xia, X.H. (2013) DAMBE5: A Comprehensive Software Package for Data Analysis in Molecular Biology and Evolution. *Molecular Biology and Evolution* , **30** , 1720-8.

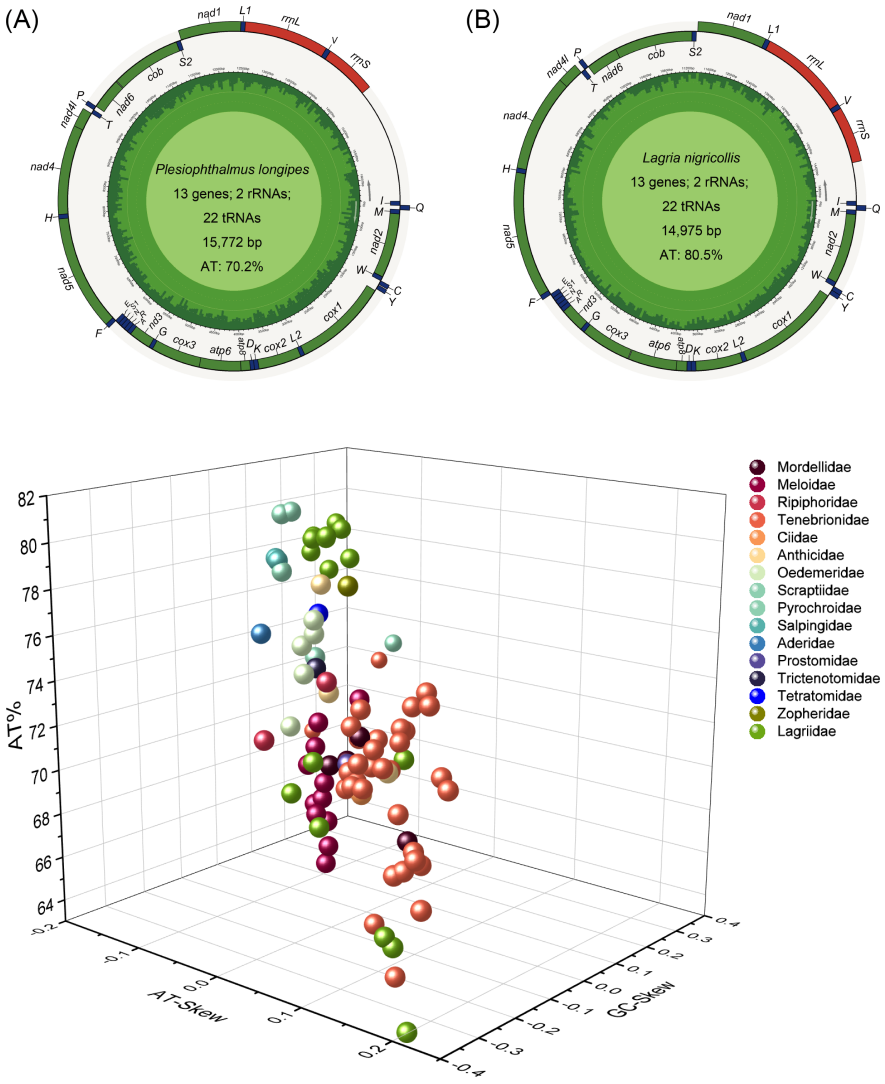
Zhang, D., Gao, F.L., Jakovlic, I., Zou, H., Zhang, J., Li, W.X. & Wang, G.T. (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources* ,**20** , 348-55.

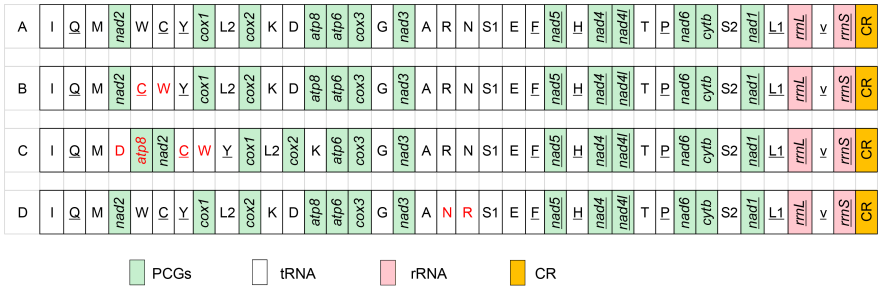
Zhang, H., Liu, N., Han, Z. & Liu, J. (2016) Phylogenetic analyses and evolutionary timescale of Coleoptera based on mitochondrial sequence.*Biochemical Systematics and Ecology* , **66** , 229-38.

Zhang, S.Q., Che, L.H., Li, Y., Dan, L., Pang, H., Ślipiński, A. & Zhang, P. (2018) Evolutionary history of Coleoptera revealed by extensive sampling of genes and species. *Nature Communications* ,**9** .

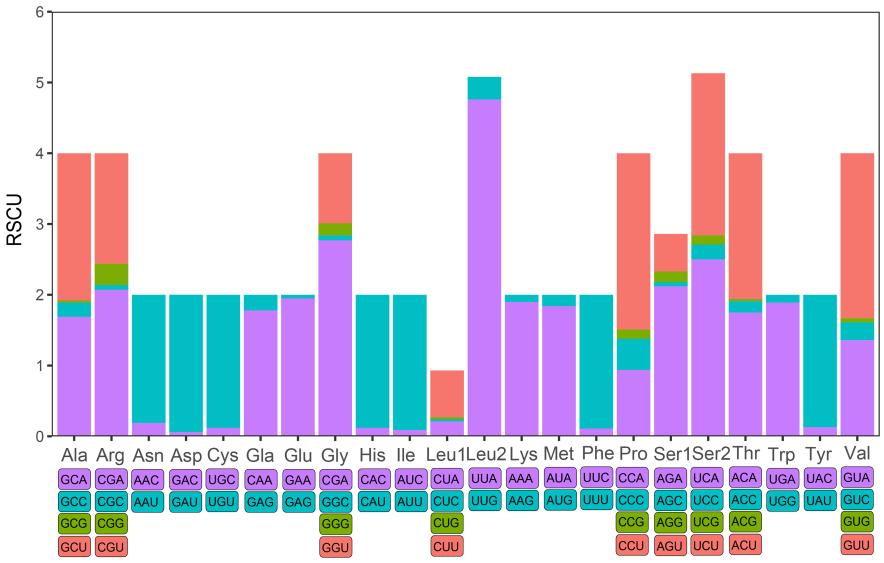
Zheng, S., Poczai, P., Hyvönen, J., Tang, J. & Amiryousefi, A. (2020) Chloroplot: An online program for versatile plotting of the organelle genomes. *Frontiers in Genetics,* **11** , 1664-8021.

Zhou, Z.C., Liu, Y.Y. & Chen, X.S. (2021) Structural Features and Phylogenetic Implications of Three New Mitochondrial Genomes of Blister Beetles (Coleoptera: Meloidae). *Journal of Insect Science* ,**21** , 6.

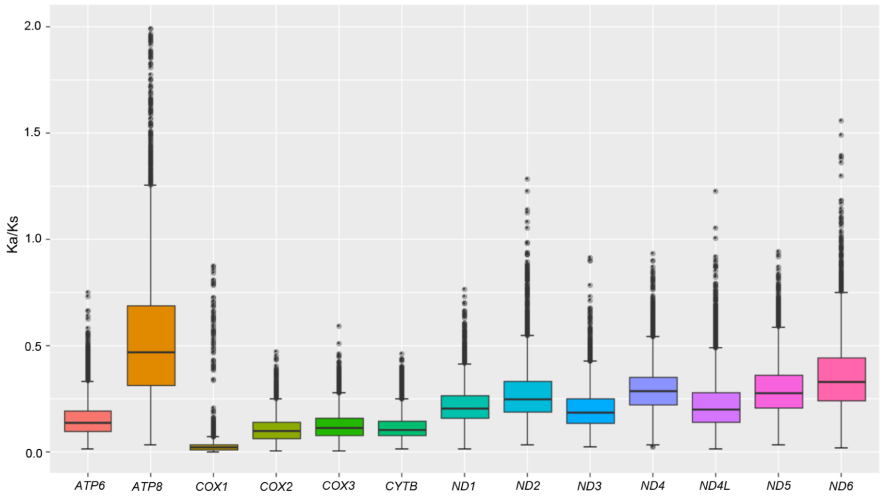


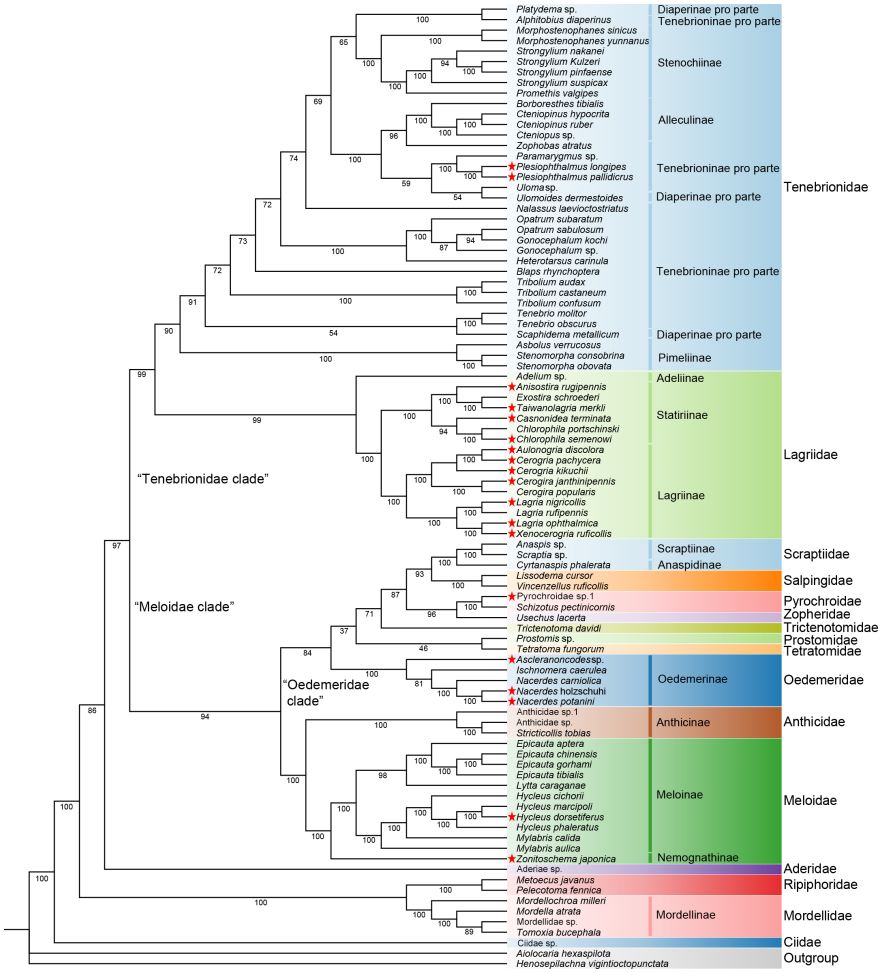


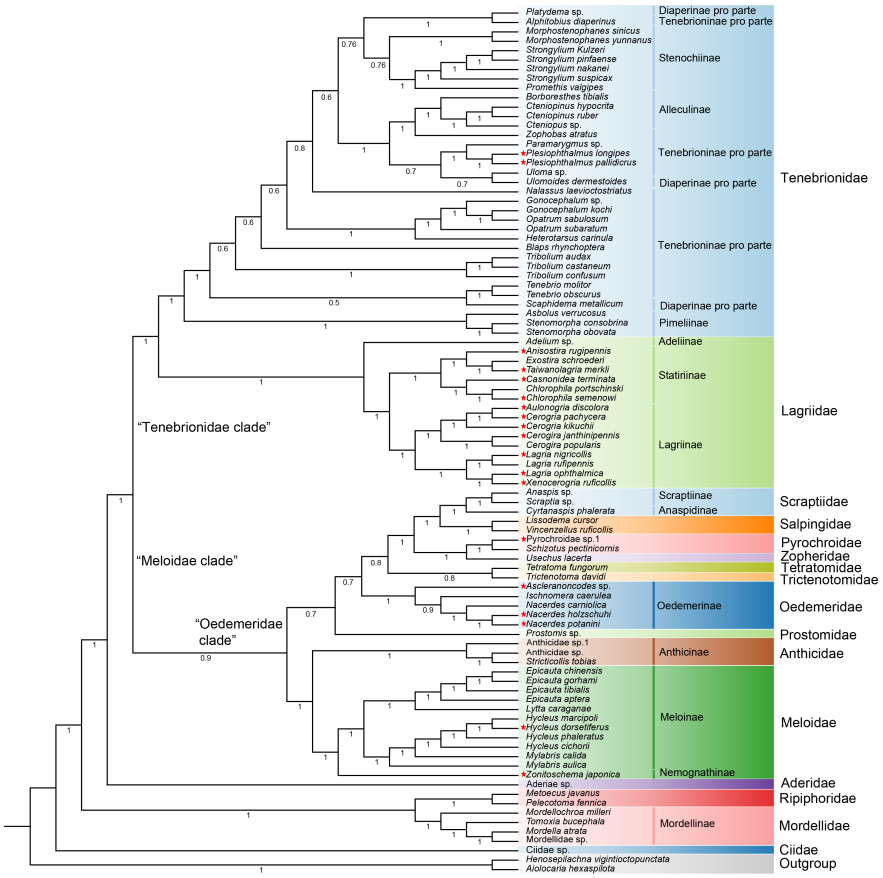
A: *Drosophila yakuba*;
B: Pyrochroidae (*Pyrochroa* sp., *Schizotus pectinicornis*);
C: Lagriidae (*Anisostira rugipennis*);
D: Mordellidae（Mordellidae sp., *Mordellochroa milleri*, *Mordella atrata, Tomoxia bucephala*).

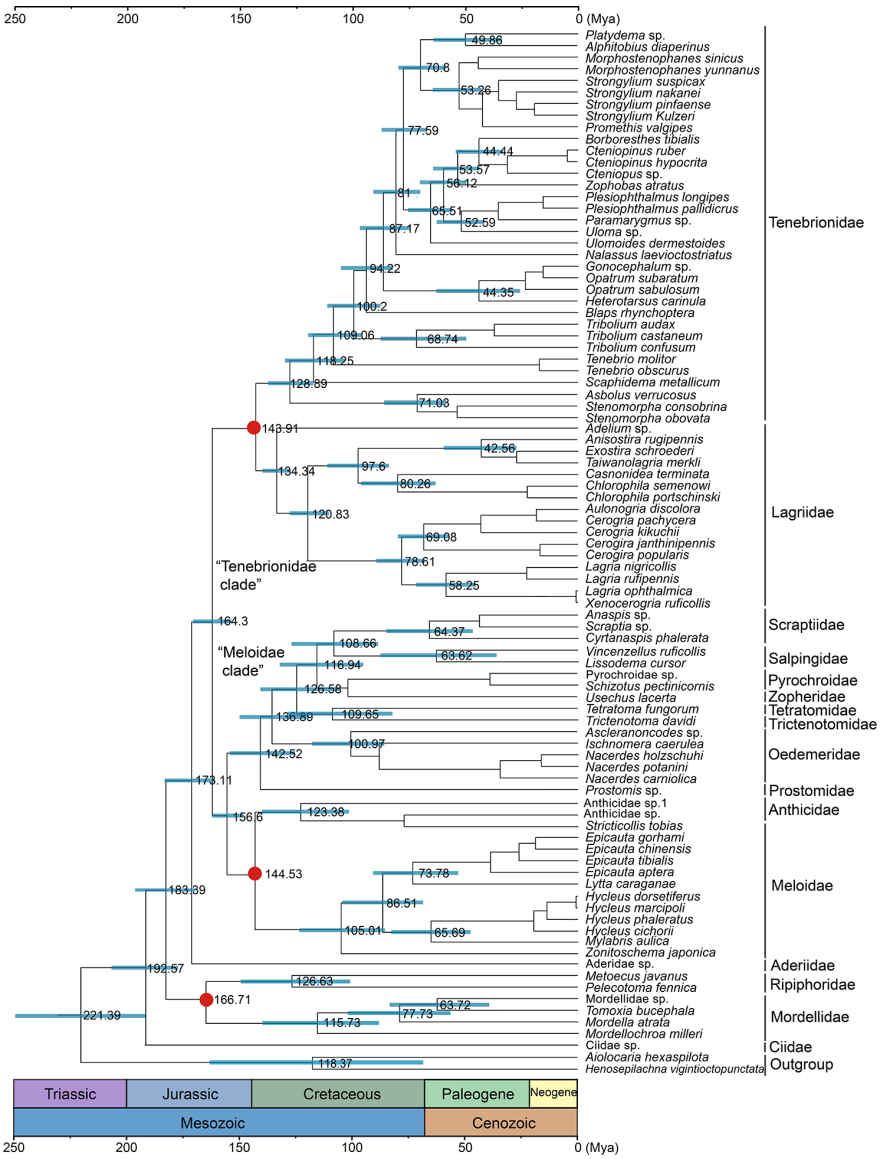


Lagria ophthalmica









Hosted file

Table 1.doc available at https://authorea.com/users/753635/articles/723818-comparative-mitogenome-research-revealed-the-phylogenetics-and-evolution-of-the-superfamily-tenebrionoidea-coleoptera-polyphage

Hosted file

Table 2.doc available at https://authorea.com/users/753635/articles/723818-comparative-mitogenome-research-revealed-the-phylogenetics-and-evolution-of-the-superfamily-tenebrionoidea-coleoptera-polyphage