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Seven new mitochondrial genomes of phytophagous scarab beetles (Coleoptera: Scarabaeidae) and phylogenetic implications

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Abstract

Among Scarabaeidae, the phytophagous scarab lineage including Melolonthinae, Cetoniinae, Dynastinae, and Rutelinae is considered important due to its members' roles as agricultural pests or pollinators. In this study, the near-complete mitochondrial genomes of seven species from six genera in the phytophagous scarab lineage were newly sequenced: *Anomala russiventris* (Fairmaire, 1893); *Apogonia* cf. *basalis* (Moser, 1915); *Apogonia splendida* (Boheman, 1858); *Coenochilus striatus* (Westwood, 1874); *Trichogomphus mongol* (Arrow, 1908); *Sophrops subrugatus* (Moser, 1921) and *Tetraserica leishanica* (Liu, Bai, Yang & Ahrens, 2014). The complete mitochondrial genomes from the 6 species include 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs), and 1 control region, which have a highly conserved gene arrangement, except for *Tr. mongol* with the rearrangement of 2 tRNA genes (*tRNA-Ile* and *tRNA-Gln*), which is a potential identified subfamily-level character of Dynastinae. In order to test whether the mitogenomic data are suited for high-level phylogenetic inferences, the substitution saturation and heterogeneity were analyzed. The results showed no sign that the phylogenetic inferences were biased by substitution saturation or the low heterogeneity of the sequence composition for most pairwise comparisons between the sequences for the entire dataset (13 PCGs) and the amino acids dataset (13 PCGs_AA). Based on the combined data of 13 PCGs and 13 PCGs_AA from the mitogenomes of 37 taxa, the phylogeny of the phytophagous lineage was explored using RAxML and Bayesian methods. The results confirmed that Cetoniinae, Rutelinae, and Dynastinae are monophyletic, and that the latter two are sister groups. Melolonthinae is a paraphyletic group, and its tribes, Diplotaxini, Euchirini, Melolonthini, Rhizotrogini, and Sericini, are a monophyletic group. The subfamily rank of Dynastinae and the tribe rank of Anomalini and Adoretini are supported.

Key words: Scarabaeidae, phytophagous scarab lineage, mitochondrial genomes, deep phylogenetic relationships

Introduction

The four subfamilies Melolonthinae, Cetoniinae, Dynastinae, and Rutelinae are known as the phytophagous scarab lineage (Erichson 1847). They are mostly plant feeders feeding on leaves, tubers, flowers, and roots, except for the larvae of the genus *Sparrmannia* Laporte, 1840, which are dung feeders (Scholtz 1988; Scholtz & Grebennikov 2016). Approximately 750 genera and 11,000 species of Melolonthinae, 200 genera and 4,100 species of Rutelinae,

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225 genera and 1,500 species of Dynastinae, and 400 genera and 3,000 species of Cetoniinae have been recorded worldwide (Scholtz & Grebennikov 2016). Some phytophagous scarabs that destroy large amounts of crops and pastures causing considerable economic losses are considered agricultural pests (Frew *et al*. 2016), while some of them play important roles as pollinators in the agricultural landscape (Mayer *et al*. 2006).

During the past decades, the phylogenetic relationships among the four subfamilies within Scarabaeidae have been explored based on morphological and molecular data. Browne & Scholtz (1998) first analyzed the phylogenetic relationships of nearly all the subfamilies of Scarabaeidae, which supported four subfamilies belonging to a single independent lineage. The clade combined by the four phytophagous scarab subfamilies is well-supported as a monophyletic lineage (Erichson 1847; Howden 1982; Browne & Scholtz 1998; Smith *et al*. 2006; Ahrens 2011, 2014; Gunter *et al*. 2016). However, despite the importance of phytophagous scarabs themselves, the internal relationships within the lineage have been poorly studied (Smith *et al*. 2006). Some critical questions remain unresolved, such as the exact position of Anomalini and Adoretini and the rank level of Anomalini and Adoretini, which may be elevated to a subfamily rank (Smith *et al*. 2006). Based on their morphological characters, Dynastinae, Cetoniinae, and Rutelinae are well-defined subfamilies, but Melolonthinae was a poorly defined subfamily (Browne & Scholtz 1998). Based on the molecular data, the phylogenetic relationship of Dynastinae and Rutelinae has not been confirmed as sister groups. The subfamily rank of Dynastinae is controversial. It is either nested in Rutelinae (Ahrens 2011, 2014) or is a sister group with Rutelinae (Ayivi *et al*. 2021).

The mitochondrial genome (mitogenome) is widely studied in insects, as a source of sequence data for phylogenetic analysis (Cameron 2014). It has been proved as a significant marker by which to infer high-level phylogenetic relationships (Nie *et al*. 2018, 2020, 2021; Li *et al*. 2020). However, mitogenomic data have some disadvantages such as recombination, faster mutation rates, and saturation (Ballard & Whitlock 2004; Rubinoff & Holland 2005). Moreover, mitochondrial genome is small and is not possible to get a well-resolved gene tree even with complete data (Ballard & Whitlock 2004). Although tree topologies were sensitive to data types and inference methods, mitogenomic data could provide useful information for resolving the Coleoptera phylogeny at various taxonomic levels by using suitable datasets and heterogeneous-site models (Yuan *et al*. 2016).

Even though the phytophagous clade of Scarabaeidae is extremely large with c.a. 20,000 described species, their mitogenomes are poorly reported (Ayivi *et al*. 2021). There are 51 mitogenomes published thus far. The limited mitochondrial data (among Pleurosticti) is not conducive to deeply analyze the phylogenetic relationships (Ayivi *et al*. 2021). In this study, we sequenced seven new mitogenomes of phytophagous scarabs. To explore the high-level relationship of phytophagous scarabs, we combined our new data with other available mitogenomes. Firstly, we analyzed the mitochondrial character of the dataset, such as substitution saturation and heterogeneity to ensure that the concatenated dataset was suitable for the inference of phylogenetic analysis. Secondly, we analyzed the high-level phylogenetic relationship using the Bayesian inference (BI) and maximum likelihood (ML) methods.

Materials and Methods

2.1. Samples and DNA Extraction

Seven specimens were collected from different parts of Hong Kong, China, during May–June 2017. The specimen of *Anomala russiventris* Fairmaire, 1893 was collected using a light trap at Pat Sin Leng Country Park (geographic coordinates: 22°49’N; 114°19’E). The specimen of *Sophrops subrugatus* (Moser, 1921) was obtained using a flight interception trap (FIT) (Nie *et al.* 2017) at Pat Sin Leng Country Park (geographic coordinates: 22°29’N; 114°10’E). The specimens of *Coenochilus striatus* (Westwood, 1874); *Trichogomphus mongol* (Arrow, 1908); *Apogonia splendida* (Boheman, 1858) and *Apogonia* cf. *basalis* (Moser, 1915) were also collected using a FIT at Ma On Shan Country Park (geographic coordinates: 22°22’N; 114°14’E). The specimen of *Tetraserica leishanica* (Liu, Bai, Yang & Ahrens, 2014) was obtained using a FIT at Ma On Shan Country Park (geographic coordinates: 22°38’N; 114°25’E). All the samples were preserved in 100% ethanol at -20°C and cataloged in the Institute of Zoology, Chinese Academy of Sciences. Genomic DNA was extracted using the DNeasy Blood & Tissue kit (QIAGEN, Germany).

2.2. DNA sequencing and mitogenome assembly and annotation

Genomic DNA was sequenced on the Illumina platform at Berry Genomics Corporation (Beijing) with a read size of 150 bp. Firstly, low-quality reads were removed, and then we assembled the remaining high-quality reads

using Spades v3.13.0 and GetOrganelle v1.7.1 (Bankevich *et al*. 2012; Jin *et al*. 2018). Secondly, the mitochondrial genomes were annotated using Geneious Prime 2020.2.4 (Kearse *et al*. 2012). The secondary structures and the anticodon of the tRNAs of the mitochondrial genomes were identified using the MITOS Web Server (http://mitos2.bioinf.uni-leipzig.de/index.py) (Bernt *et al.* 2013) and tRNAscan-SE v1.3.1 (Schattner *et al*. 2005). Thirdly, codon usage and relative synonymous codon usage (RSCU) were analyzed using MEGA v.7.0 (Kumar *et al*. 2016) and graphically drawn using ggplot2 and aplot packaging in R (Wickham 2009). The map of the mitogenomes was drawn using the CGView Server (http://cgview.ca) (Grant & Stothard 2008). Lastly, we calculated the strand asymmetry using the formulas: AT-skew = [A-T]/[A+T] and GC-skew = [G-C]/[G+C].

2.3. Phylogenetic analyses

The mitogenomes of 7 species were newly (GenBank accession Nos: MW829593—MW829599)) (Note: *Te. leishanica* was not used to infer the phylogeny.) and 42 additional mitogenomes (deleted 12 repeats or bad quality mitogenomes) from 51 available mitogenomes obtained from the National Center for Biotechnology Information (NCBI) (Bethesda, MD, U.S.A.) were used for phylogenetic analyses (Table 1).

TABLE 1. Examined species and respective NCBI accession numbers including reference information.

Subfamily	Tribe	Species	Acc. No.	Length (bp)	References
Cetoniinae	Cetoniini	*Leucocelis* sp.	JX412740	14,328	Timmermans *et al.* (2016)
Cetoniinae	Cetoniini	*Protaetia brevitarsis*	MN418316	17,783	Choi *et al*. (2020)
Cetoniinae	Cetoniini	*Protaetia speciosa*	OK484307	16,955	Ayivi *et al*. (2021)
Cetoniinae	Cremastocheilini	*Coenochilus striatus*	MW829597	15,480	this study
Cetoniinae	Goliathini	*Dicronocephalus adamsi*	OK012569	18,550	unpublished
Cetoniinae	Goliathini	*Dicronorhina derbyana*	OK484300	16,609	Ayivi *et al*. (2021)
Cetoniinae	Goliathini	*Eudicella quadrimaculata*	OK484299	16,690	Ayivi *et al*. (2021)
Cetoniinae	Goliathini	*Eudicella smithii*	OK484302	16,712	Ayivi *et al*. (2021)
Cetoniinae	Goliathini	*Eudicella tetraspilota*	OK484301	18,302	Ayivi *et al*. (2021)
Cetoniinae	Goliathini	*Goliathus goliatus*	OK484303	18,699	Ayivi *et al*. (2021)
Cetoniinae	Goliathini	*Jumnos ruckeri*	OK484304	19,468	Ayivi *et al*. (2021)
Cetoniinae	Goliathini	*Mecynorhina polyphemus*	OK484305	16,422	Ayivi *et al*. (2021)
Cetoniinae	Goliathini	*Mecynorhina torquata*	OK484306	17,192	Ayivi *et al*. (2021)
Cetoniinae	Osmodermatini	*Osmoderma opicum*	KU500641	15,341	Kim *et al*. (2016)
Cetoniinae	Trichiini	*Myodermum* sp.	JX412847	12,950	Timmermans *et al*. (2016)
Dynastinae	Dynastini	*Chalcosoma caucasus*	OK484308	19,444	Ayivi *et al*. (2021)
Dynastinae	Dynastini	*Dynastes hercules*	OK484309	17,813	Ayivi *et al*. (2021)
Dynastinae	Dynastini	*Megasoma elephas*	OK484310	16,785	Ayivi *et al*. (2021)
Dynastinae	Dynastini	*Megasoma mars*	OK484311	16,983	Ayivi *et al*. (2021)
Dynastinae	Dynastini	*Xylotrupes beckeri*	OK484313	18,567	Ayivi *et al*. (2021)
Dynastinae	Dynastini	*Xylotrupes socrates*	OK484315	18,660	Ayivi *et al*. (2021)
Dynastinae	Dynastini	*Xylotrupes sumatrensis*	OK484316	19,687	Ayivi *et al*. (2021)
Dynastinae	Oryctini	*Oryctes nasicornis*	OK484312	20,396	Ayivi *et al*. (2021)
Dynastinae	Oryctini	*Oryctes rhinoceros*	MT457815	20,898	Filipović *et al*. (2021)
Dynastinae	Oryctini	*Trichogomphus mongol*	MW829599	16,737	this study
Dynastinae	Phileurini	*Eophileurus chinensis*	MW632132	16,624	Cheng *et al*. (2021)

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TABLE 1. (Continued)

Subfamily	Tribe	Species	Acc. No.	Length (bp)	References
Melolonthinae	Diplotaxini	*Apogonia* cf. *basalis*	MW829596	15,226	this study
Melolonthinae	Diplotaxini	*Apogonia splendida*	MW829595	16,728	this study
Melolonthinae	Euchirini	*Cheirotonus gestroi*	MN893347	16,899	Yang *et al*. (2020)
Melolonthinae	Euchirini	*Cheirotonus jansoni*	KC428100	17,249	Shao *et al*. (2014)
Melolonthinae	Melolonthini	*Melolontha hippocastani*	KX087316	15,485	unpublished
Melolonthinae	Melolonthini	*Polyphylla gracilicornis*	MW143080	16,793	Zhou *et al*. (2021)
Melolonthinae	Melolonthini	*Polyphylla laticollis*	KF544959	14,473	Kim *et al*. (2013)
Melolonthinae	Melolonthini	*Rhopaea magnicornis*	FJ859903	17,522	Cameron *et al*. (2009)
Melolonthinae	Rhizotrogini	*Amphimallon solstitiale*	MH899179	13,755	Yang *et al*. (2019)
Melolonthinae	Rhizotrogini	*Eotrichia niponensis*	MZ726798	16,851	unpublished
Melolonthinae	Rhizotrogini	*Pedinotrichia parallela*	MW874410	16,975	unpublished
Melolonthinae	Rhizotrogini	*Sophrops subrugatus*	MW829598	16,409	this study
Melolonthinae	Sericini	*Pleophylla* sp.	JX412736	12,579	Timmermans *et al*. (2016)
Melolonthinae	Sericini	*Serica* sp.	MF997050	13,815	Song & Zhang (2018)
Rutelinae	Adoretini	*Adoretus* sp.	JX412788	12,581	Timmermans *et al*. (2016)
Rutelinae	Anomalini	*Anomala russiventris*	MW829593	15,601	this study
Rutelinae	Anomalini	*Mimela splendens*	MZ064554	15,148	Unpublished
Rutelinae	Anomalini	*Popillia japonica*	MG971231	16,541	Yang *et al*. (2018)
Rutelinae	Anomalini	*Popillia mutans*	MF997049	16,192	Song & Zhang (2018)
Scarabaeinae	Oniticellini	*Euoniticellus fulvus*	KU739453	15,494	Breeschoten *et al*. (2016)
Scarabaeinae	Onitini	*Cheironitis hoplosternus*	KU739450	14,924	Breeschoten *et al*. (2016)
Scarabaeinae	Onthophagini	*Caccobius nigritulus*	KU739484	15,039	Breeschoten *et al*. (2016)

Assessment of sequence variation

Based on 48 taxa, the phylogenetic relationship of the phytophagous groups was inferred from the nucleotides and amino acids of all the PCGs. All the protein genes were aligned using MAFFT v.7 with the genetic code of invertebrate mitochondria (Katoh & Standley 2013). The gaps and ambiguous sites were filtered using Gblocks 0.91b (Castresana 2000) invoked by Phylosuite v.1.2.2 (Zhang *et al*. 2020), with the default parameters. The aligned sequences for each locus were concatenated using the Concatenate Sequence function of the Phylosuite platform (Zhang *et al*. 2020). Substitution saturation was assessed using DAMBE v.7 with the GTR model (Xia 2018). The heterogeneity of the sequence variation, using a nucleotides dataset and an amino acid dataset of 13 PCGs, was assessed with AliGROOVE (Kück *et al*. 2014) with the default sliding window size. The partitioning schemes were analyzed using PartitionFinder2 (Lanfear *et al*. 2016).

Phylogenetic analyses

Two datasets (13 PCGs and 13 PCGs_AA) were used to infer phylogenetic trees in MrBayes v3.2.7 (Ronquist *et al*. 2012) with 48 taxa. We also conducted the Markov Chain Monte Carlo (MCMC) with the best-fitting models of sequence evolution. The MrBayes runs were performed in CIPRES (http://www.phylo.org) (Miller *et al*. 2010). The MCMC searches were conducted for 30,000,000 generations with a random starting tree, and the samples were obtained every 1,000 generations. The first 10% of generations were discarded as burn-ins. The chains were stopped when the average standard deviation of the split frequencies fell below 0.01. The convergence of the parameters and stationarity of the runs were checked in Tracer v.1.7 (Rambaut *et al*. 2018), and all the parameters had an effective sample size (ESS) of over 200. The maximum likelihood (ML) search was carried out in RAxML v8.0.26 (Stamatakis 2014) for 1,000 rapid bootstrap replicates. The nucleotides dataset used the GTRGAMMA model, and the amino acids dataset used the PROTGAMMAIMTZOAF model.

Results

3.1. Mitogenome organization and composition

The mitogenomes of 7 species were obtained: *An. russiventris* (15,601 bp), *Ap. splendida* (16,728 bp), *Ap.* cf. *basalis* (15,226 bp), *S. subrugatus* (16,409 bp), *C. striatus* (15,480 bp), and *Tr. mongol* (16,737 bp), which contained 37 genes (13 PCGs, 22 tRNAs, 2 rRNAs) and a large non-coding region (Control Region, CR); *Te. leishanica* (10,031 bp) included 26 genes with 11 genes missing (Figure 1). There is a strong bias of the total nucleotide composition of all the species' mitogenomes toward A and T. Overall, the nucleotide composition and the genome structure of the seven species showed features typical of Scarabaeidae mitogenomes. The information regarding the A+T content of other species is shown in Table 2.

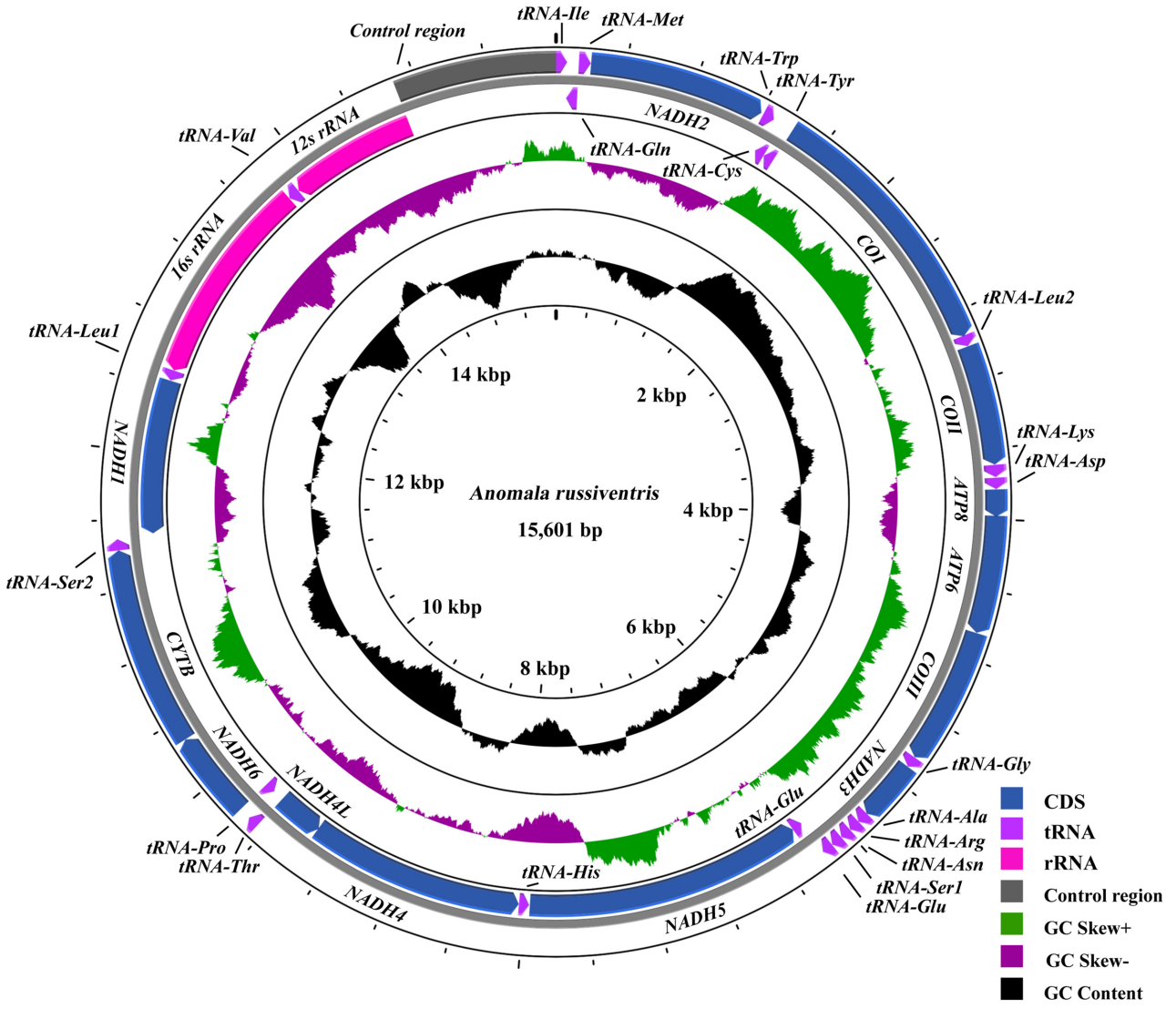

FIGURE 1. Map of the *Anomala russiventris* mitogenome.

The total length of 13 PCGs in the 6 mitogenomes ranges from 11,132 bp to 11,144 bp, except *Te. leishanica*, which is 8,111 bp in length. The details of start and stop codons of protein-coding genes in seven new mitogenomes are shown in Table 3. The relative synonymous codon usage (RSCU) of the six species is highly similar to that of beetles, and reflects a codon usage bias (Sharp & Li 2018; Yu *et al*. 2019) (Figure 2). The 16S rRNA gene is located between *tRNA-Leu* (TAG) and *tRNA-Val*. The 12S rRNA gene is located between *tRNA-Val* and the control region. The location and characteristics of the two rRNA genes are similar to those of other beetles. The order of the tRNA genes in most beetles is very conserved. In the mitogenome of *Tr. mongol*, the order of *tRNA-Ile* and *tRNA-Gln* is

reversed, and this phenomenon can be found in the *Oryctes rhinoceros* mitogenome (Filipović *et al*. 2021). All the 22 tRNAs are folded into the typical cloverleaf structures, except *tRNA-Ser1* (AGN). *tRNA-Ser1* (AGN) lacks the DHU-stem, with some kind of unmatched base pairs in the anticodon stem (Figure 3), as compared with the typical cloverleaf structures. The intergenic regions of *C. striatus*, 148 bp in length between *tRNA-Trp* and *tRNA-Cys*, are different from those of other species as a non-coding region (NCR). The two non-coding regions were discovered in *Tr. mongol*, between *tRNA-Ser2* and *NAD1* (374 bp) and between *tRNA-Gln* and *tRNA-Ile*; the former appeared in *Popillia* sp. (175 bp, JX412777), and the latter was discovered in all the species of Dynastinae. The seven new mitochondrial structures are described in more detail in Supplementary Data.

TABLE 2. Nucleotide composition of seven mitogenomes.

Species	Whole mitogenome	Protein-coding genes			*12S rRNA* genes	*16S rRNA* genes	Control region
	A+T%	A+T%	AT-skew	GC-skew	A+T%	A+T%	A+T%
An. russiventris	74.69	73.88	-0.138	-0.034	72.64	77.80	78.89
Ap. cf. *basalis*	77.27	76.33	-0.139	-0.018	77.47	80.50	87.66
Ap. splendida	74.80	73.31	-0.124	-0.068	76.33	77.16	80.19
C. striatus	73.04	72.20	-0.135	-0.042	74.71	77.81	72.14
S. subrugatus	71.64	70.83	-0.156	-0.036	71.67	75.55	71.67
Te. leishanical	75.23	74.41	-0.130	-0.034	--	--	81.75
Tr. mongol	74.46	73.76	-0.137	-0.025	72.51	77.21	75.10

TABLE 3. The details of start and stop codons of protein-coding genes in seven new mitogenomes.

Gene	*An. russiventris*	*Ap. splendida*	*Ap.* cf. *basalis*	*C. striatus*	*S. subrugatus*	*Te. leishanical*	*Tr. mongol*
	start/stop codon	start/stop codon	start/stop codon	start/stop codon	start/stop codon	start/stop codon	start/stop codon
NAD2	ATG/TAA	ATT/TAA	ATG/TAA	ATT/TAA	ATT/TAA	ATT/TAA	ATC/TAA
COI	AAT/TAA	AAC/TAA	AAT/TAA	ATT/TAA	AAT/TAA	AAT/TAA	ATT/TAA
COII	ATT/T-	ATC/T-	ATG/T-	ATT/T-	ATA/T-	ATT/T-	ATC/T-
ATP8	ATT/TAA	ATT/TAA	ATT/TAA	ATT/TAA	ATT/TAA	ATT/TAA	ATT/TAA
ATP6	ATG/T-	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	GTG/TAA
COIII	ATG/T-	ATG/T-	ATG/T-	ATG/T-	ATG/T-	ATG/T-	ATG/T-
NAD3	ATT/TAG	ATT/TAG	ATC/TAG	ATC/TAG	ATC/TAG	ATG/TAG	ATC/TAG
NAD5	ATT/TAG	ATT/TAA	ATT/TAA	ATT/TAA	ATT/TAA	ATT/TAA	ATT/T-
NAD4	ATA/T-	ATA/TAA	ATA/TAA	ATA/TAA	ATA/TAA	ATA/TAG	ATA/TAA
NAD4L	ATG/TAA	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA	--	ATG/TAA
NAD6	ATC/TAA	ATC/TAA	ATA/TAA	ATT/TAA	ATC/TAA	--	ATT/TAA
CYTB	ATG/TAG	ATG/TAG	ATG/TAG	ATG/TAG	ATG/TAG	--	ATG/TAG
NAD1	ATT/TAG	ATC/TAA	ATT/TAA	ATT/TAG	ATT/TAA	--	ATT/TAA

3.2. Phylogenetic analyses

Assessment of sequence variation

The substitution saturation of 2 rRNAs, each PCG, and the concatenated 13 PCGs dataset was assessed using DAMBE v.7 with the substitution saturation test (Xia 2018). All the result analyses showed a lower ISS (simple index of substitution saturation) value than that of the ISS.c (critical ISS value) ($P \leq 0.05$), which confirmed the suitability of the nucleotide unsaturation of 13 PCGs for phylogenetic analyses. The two datasets had low heterogeneity, and the concatenated nucleotide dataset had a higher heterogeneity than that of the amino acid dataset (Figure 5).

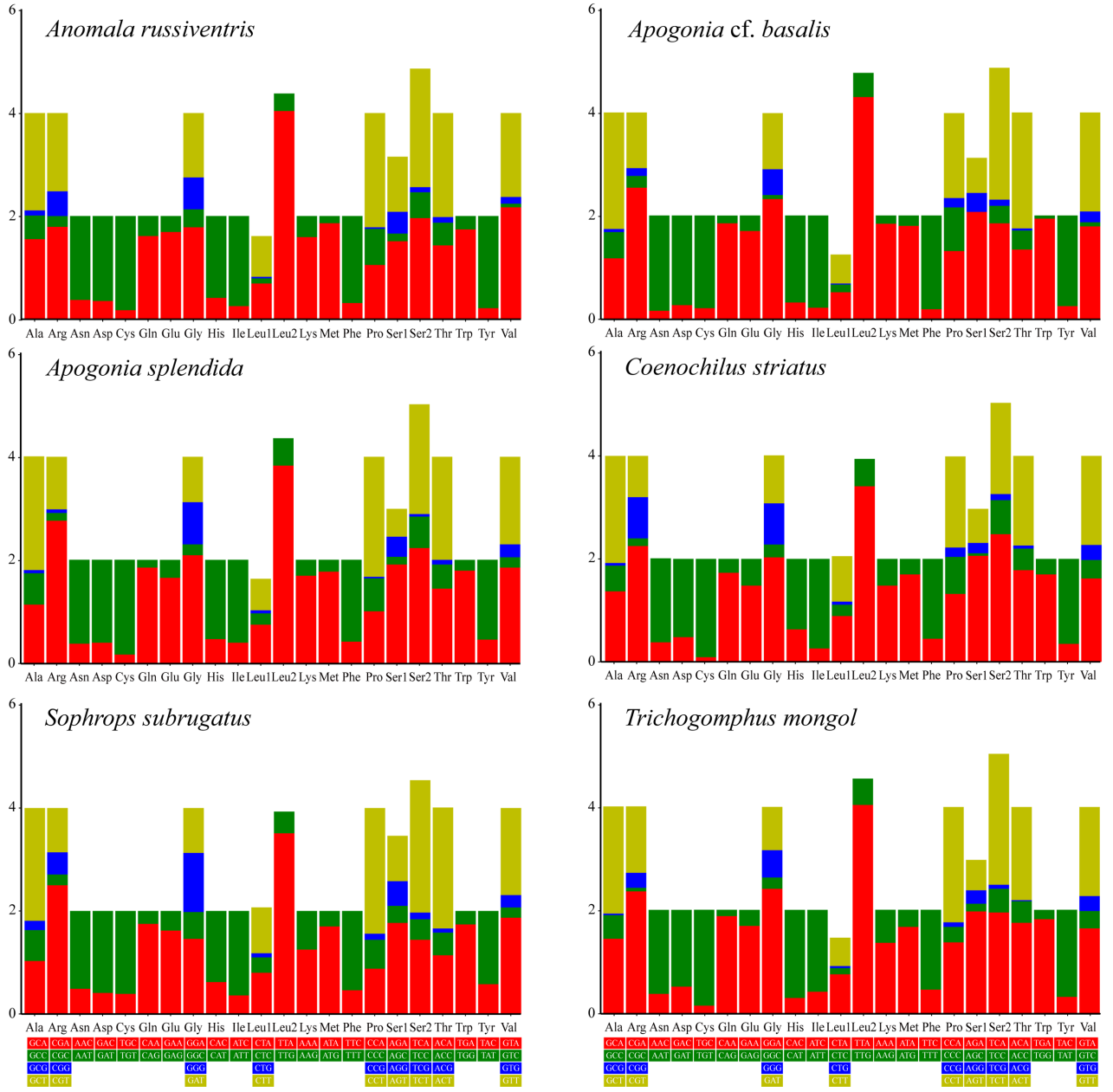

FIGURE 2. Relative synonymous codon usage (RSCU) of six mitogenomes.

The high-level phylogenetic relationship of the phytophagous scarab lineage

Based on the two datasets (13 PCGs and 13 PCGs_AA), the inferred phylogenetic trees, established using the RAxML and MrBayes methods, had similar topologies (Figure 6 and Figure 7). The three subfamilies were recovered as monophyletic groups with high supporting values: Cetoniinae (PP: 1, BS: 100), Dynastinae (PP: 1, BS: 100), and Rutelinae (PP: 1, BS: 100). Dynastinae and Rutelinae together formed a monophyletic clade (PP: 1, BS: 100) showing the closest relationship. The Dynastinae + Rutelinae clade was recovered as a sister group to the Cetoniinae clade (PP: 1, BS: 100).

The subfamily Melolonthinae was not recovered as monophyletic. Melolonthinae was divided into three clades by the nucleotide dataset: Sericini (PP: 1, BS: 100), Diplotaxini + Euchirini (PP: 1, BS: 86), and Melolonthini + Rhizotrogini (PP: 1, BS: 100), and into two clades by the amino acid dataset: Euchirini (Diplotaxini + Sericini) (BS: 100) and Melolonthini + Rhizotrogini (BS: 100) in RAxML. The five tribes in Melolonthinae were recovered as monophyletic groups, respectively: Sericini, Diplotaxini, Euchirini, Melolonthini, and Rhizotrogini. The tribe Anomalini of Rutelinae was recovered as monophyletic (PP: 1, UB: 100). Cetoniini was paraphyletic with the inclusion of *Osmoderma opicum* (Lewis, 1887); additionally, Goliathini, Dynastini, and Oryctini were recovered as non-monophyletic.

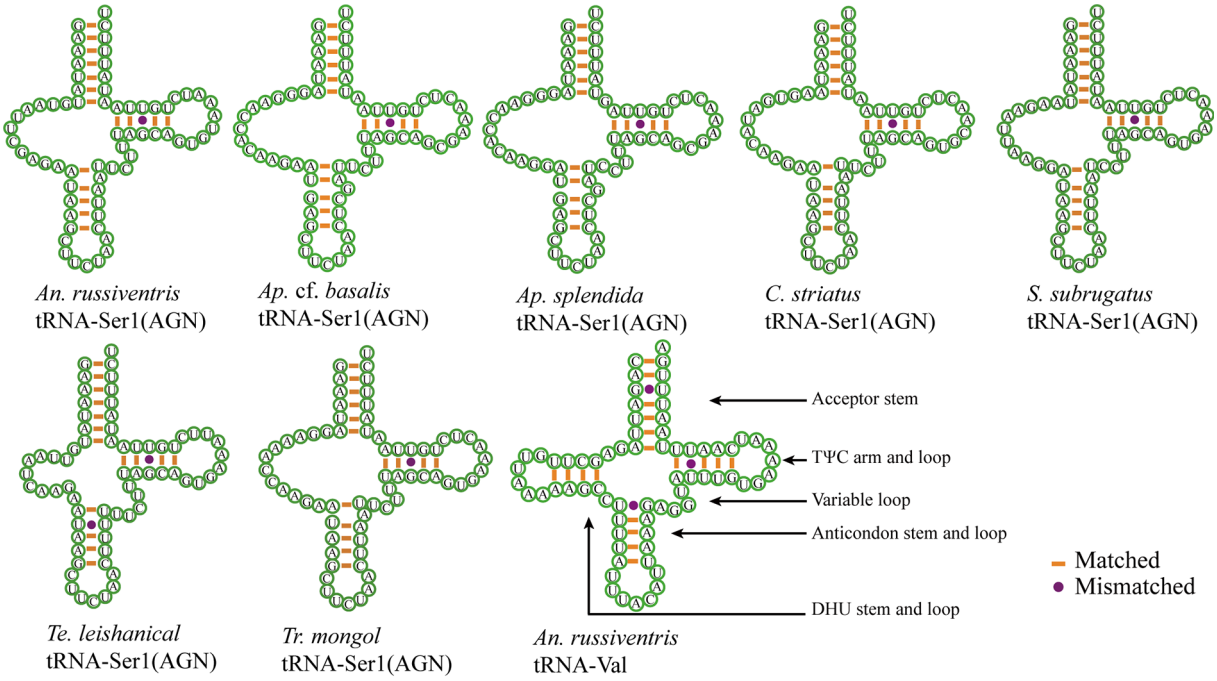

FIGURE 3. Inferred secondary structure of *tRNA-Ser1* (AGN) in seven new mitogenomes and *tRNA-Val* in the *An. russiventris* mitogenome.

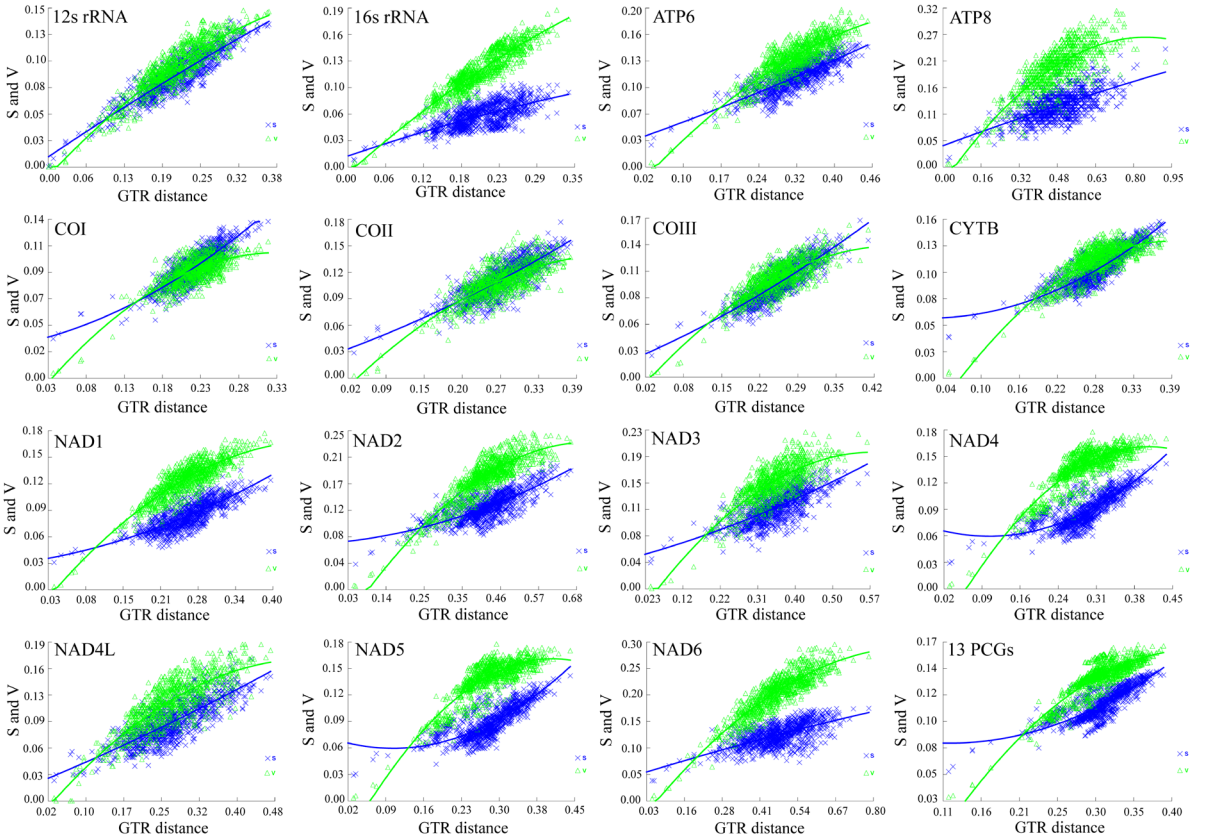

FIGURE 4. Saturation plots for 2 rRNA gens, 13 protein-coding genes, and a concatenated dataset (from 13 protein-coding genes), left to right. The plot shows uncorrected pairwise divergences in transitions (s) and transversions (v) against divergences calculated with the GTR model. Green, transversions; blue, transitions.

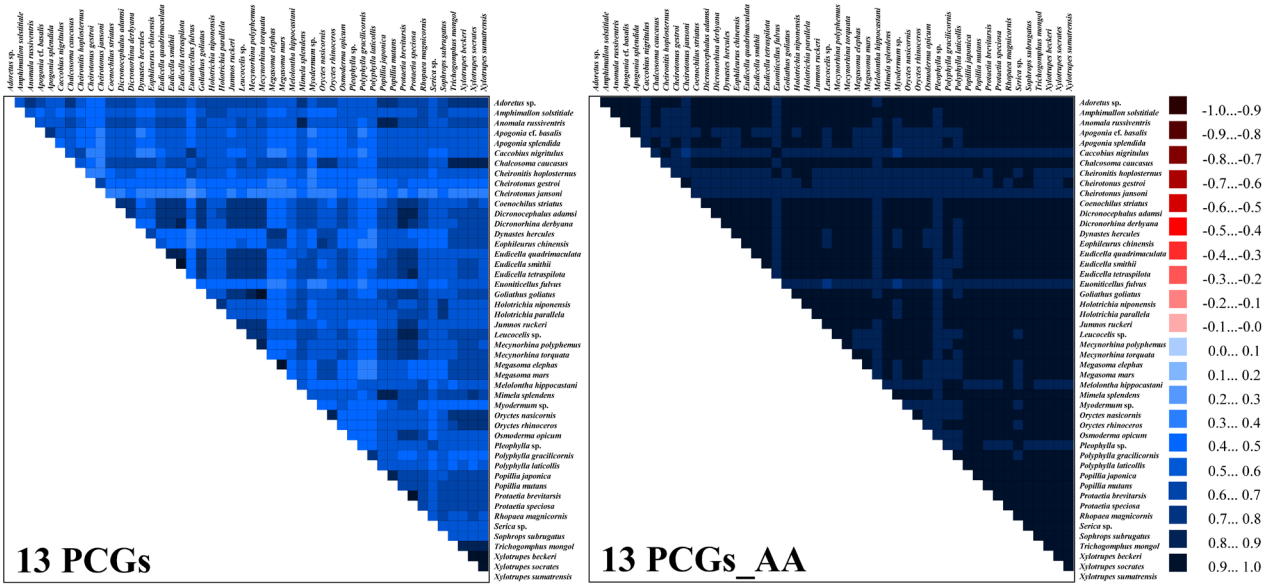

FIGURE 5. Heterogeneous sequence divergence with nucleotides dataset and amino acids dataset of 13 PCGs of all taxa. The pairwise Aliscore scores are represented by colored squares. The scores range from -1, indicating full random similarity (dark blue), to +1, indicting non-random similarity (bright orange).

Discussion

In this study, we report seven new mitogenomes of phytophagous scarabs (Rutelinae, Cetoniinae, Melolonthinae, and Dynastinae). The general features of the seven new mitogenomes were analyzed. Among them, the arrangement of the genes of six mitogenomes was sufficiently conserved. The gene order rearrangement of *tRNA-Ile* and *tRNA-Gln*, found in the sequenced *Tr. mongol*, was also discovered in all the published mitogenomes of Dynastinae, but not in the other Scarabaeidae species. Gene rearrangement was rare; however, the non-coding regions were relatively common in the phytophagous scarab lineage. The non-coding region that appeared between *tRNA-Gln* and *tRNA-Ile* only appeared in Dynastinae, but other non-coding regions showed no uniformity. The uniform translocation of *tRNA-Ile* and *tRNA-Gln* provided evidence for the monophyly of Dynastinae (Ayivi *et al*. 2021), which is a potential identified subfamily-level character of Dynastinae. It is helpful to understand the phylogenetic relationship by analyzing the structural characteristics of mitochondrial genome.

Our phylogenetic analyses results were largely consistent with previous results regarding high-level phylogenetic relationships among phytophagous scarabs: the monophyly of the phytophagous scarab beetles and the non-monophyly of the subfamily Melolonthinae (Ahrens 2006; Coca-Abia 2007; Ahrens & Vogler 2008; Ahrens *et al*. 2011, 2014; McKenna *et al*. 2015; Gunter *et al*. 2016; Yang *et al*. 2018).

The relationship between Melolonthinae tribes has been disputed (Coca-Abia 2007). The non-monophyly of Melolonthinae, recovered in several phylogenies with exoskeletal morphological characters (Ahrens 2006; Coca-Abia 2007), was supported based on two datasets. The internal phylogeny Melolonthinae showed different phylogenetic topologies in the placement of Sericini, Diplotaxini, and Euchirini according to the two datasets (13 PCGs and 13 PCGs_AA). Additionally, the tribe rank of Sericini is controversial. Some researchers supported Sericini being an independent subfamily according to the characters of the phallobase and metacoxa (Ahrens 2006; Coca-Abia 2007), but some researchers traditionally treated it as a tribe of the subfamily Melolonthinae (Browne & Scholtz 1998). In this study, Melolonthinae was poorly defined, and no consistent classification was applied (Scholtz & Grebennikov 2016).

The monophyletic relationship of the ruteline subgroup (Cetoniinae + Rutelinae + Dynastinae) was successively confirmed, mainly by the morphological characters of the hindwing articulation, wing base, and molecular datasets (Browne & Scholtz 1998, Smith *et al*. 2006; Ahrens *et al*. 2011; McKenna *et al*. 2015; Gunter *et al*. 2016). The unclear phylogenetic relationship between Dynastinae and Rutelinae was further analyzed. Our, and others', mitogenomic phylogenetic analyses confirmed the subfamily rank of Dynastinae and the tribe rank of Anomalini and Adoretini

with high supporting values (Ayivi *et al*. 2021), which differs from the viewpoint of other scholars (Browne & Scholtz 1998, Smith *et al*. 2006; Ahrens *et al*. 2011; McKenna *et al*. 2015; Gunter *et al*. 2016). We performed whole mitogenome sequences of Dynastinae to advance the understanding of the ruteline subgroup relationship (Scholtz & Grebennikov 2016; Song & Zhang 2018).

We do not propose any taxonomic treatment herein because a more extensive systematic approach with thorough taxon sampling, including type series, large genomic data, and morphological characters examined from different life stages, is needed.

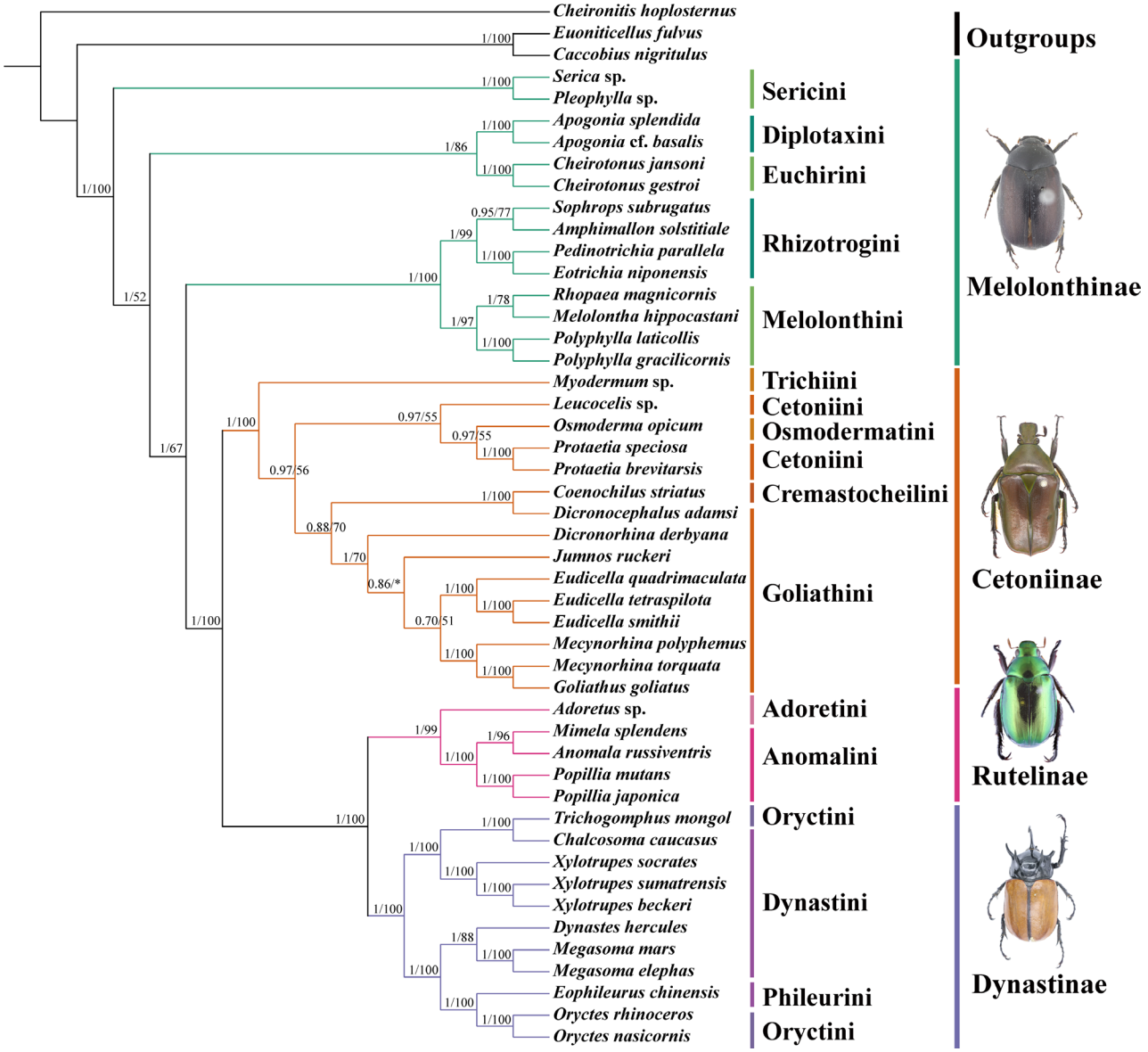

FIGURE 6. Phylogenetic tree produced using maximum likelihood (ML) and Bayesian (BI) methods based on the nucleotide sequences of 13 PCGs. The numbers on the left are Bayesian posterior probabilities (PP), and those on the right are maximum likelihood bootstrap values (BS). Asterisk indicates that this node is different in ML and BI.

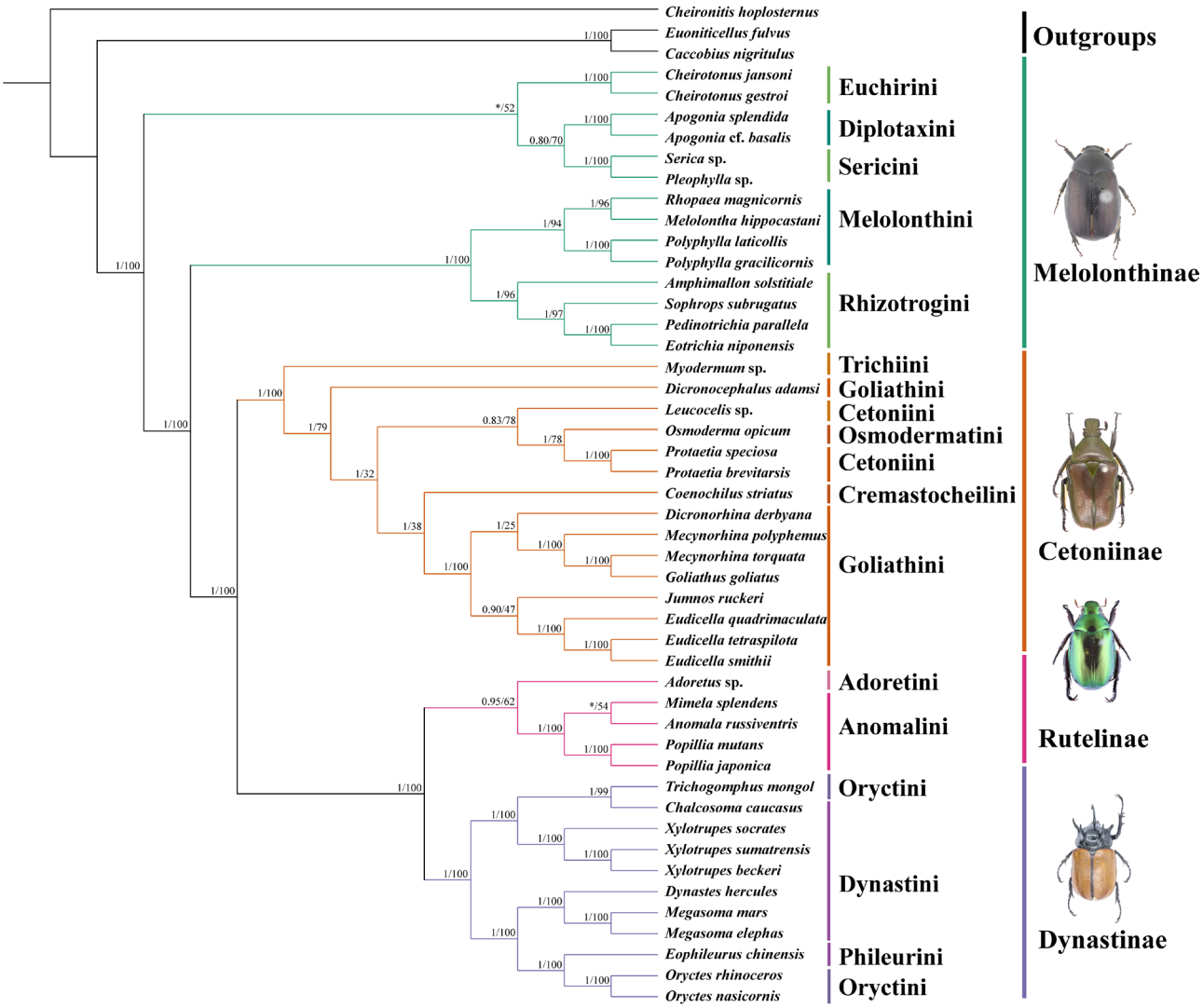

FIGURE 7. Phylogenetic tree produced using maximum likelihood (ML) and Bayesian (BI) methods based on amino acids of 13 PCGs. The numbers on the left are Bayesian posterior probabilities (PP), and those on the right are maximum likelihood bootstrap values (BS). Asterisk indicates that this node is different in ML and BI.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

All authors reviewed and approved the final manuscript.

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