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MITOGENOME REPORT

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The complete mitochondrial genome of the shining leaf chafer *Mimela junii* (Duftschmidt, 1805) (Coleoptera: Scarabaeidae)

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ABSTRACT

The complete mitochondrial genome of the shining leaf chafer *Mimela junii* was sequenced and is herein described. The mitogenome consists of a circular molecule of 16,805 bp, with an overall AT content of 75.7%. It encodes for 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs) and contains a non-coding Control Region (CR) characterized by the presence of tandem repeats. The gene order corresponds to the ancestral Pancrustacea model and mitogenome characteristics are congruous with those of hexapods. In the phylogenetic analysis, *M. junii* is nested within a paraphyletic *Anomala* with high support, and is herein associated with *Anomala corpulenta* with medium/low support.

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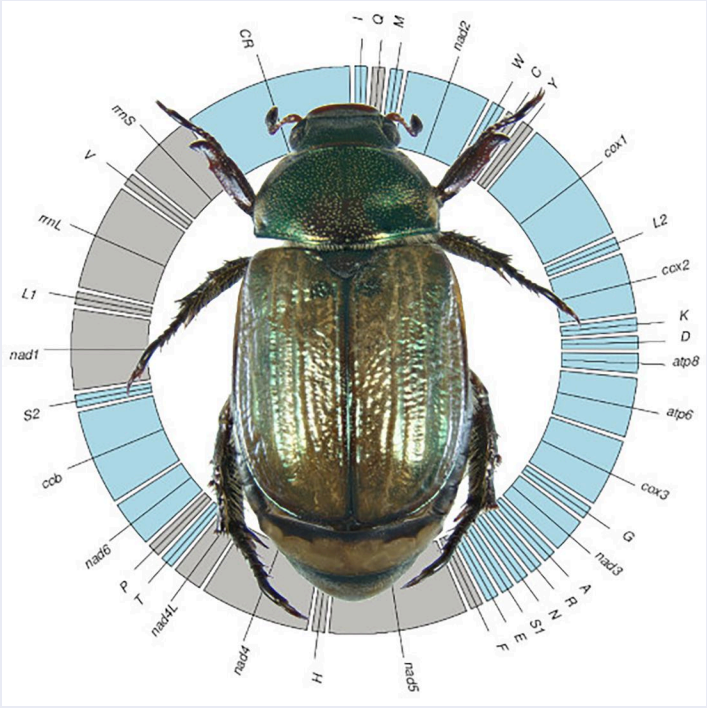
Rutelinae; *Anomala*; mitogenomics; molecular identification; agricultural pest

GRAPHICAL ABSTRACT



Introduction

Mimela junii (Duftschmidt, 1805) (Coleoptera: Scarabaeidae; Figure 1), belongs to the diverse sub-family Rutelinae (shining leaf chafers) (Jameson et al. 2003; Ballerio et al. 2014). *M. junii* is a fairly common species, widely distributed in Central and Southern Europe (Baraud 1992), and is usually associated with sandy soils, both near the coasts and in the interior. The larvae live in the soil and are rhizophagous, while the summer-flying adults feed on leaves of spontaneous shrubs and grasses (Ballerio et al. 2014).

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Sequencing of the complete mitochondrial genome of *M. junii* was performed to aid molecular identification within shining leaf chafers, that include notable pests, e.g. *Popillia japonica*, whose morphological identification is often problematic, especially at the larval stage. This is the first complete mitochondrial genome for the species. A second complete mitochondrial genome is available in the NCBI database for the co-generic species *Mimela splendens* (MZ064554, unpublished); complete (or semi complete) mitochondrial genomes are available in NCBI for eight additional species from sub-family Rutelinae.

Materials and methods

Larvae of *M. junii* were manually collected (Siena, Italy: 43.337833 N, 11.336306E) and reared until adult emergence. Species attribution was confirmed by morphological analysis of a set of adult diagnostic characters, i.e. morphology of antenna, clypeus, pronotum and tarsus (Baraud 1992; Ballerio et al. 2014), as well as barcoding (Ratnasingham and Hebert 2007).

All DNA from one individual was used for sequencing. A second specimen, as well as the DNA of a third, were deposited in the collection of the Department of Life Sciences (URL: www.dsv.unisi.it, contact: D. Badano, davide.badano@unisi.it) under vouchers MJU6_DB and MJU7_DB_DNA, respectively.

Figure 1. *Mimela junii*, adult habitus. Photo by D.B.

DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Sequencing (TruSeq DNA PCR-Free) was performed at Macrogen Europe (The Netherlands) on a Novaseq6000 (Illumina) applying a 150 bp PE layout to produce a total of ~332 million PE reads. Sequences were trimmed in fastp (ver. 0.23.2; Chen et al. 2018). Reads were assembled *de novo* in MegaHit (ver. 1.2.9; Li et al. 2015), under default settings, as well as in NovoPlasty (ver. 4.3.4; Dierckxsens et al. 2017), under multiple k-mer lengths (33 to 143) using the *cox1* sequence from the MegaHit assembly as the seed. MegaHit produced a contig that was manually circularized based on terminal overlaps. NovoPlasty produced circular genomes ($k = 33$, 55, 121) or linear contigs that could be readily circularized based on terminal overlaps ($k = 77$, 99, 111). All assemblies were identical in sequence apart for a short segment of the CR (~376 bp) containing tandem repeats. The sequence obtained with $k = 121$ (the longest k that circularized automatically) was selected as the most reliable and is herein described. Coverage was assessed in bbmap ver. 15/9/2022 (Brian Bushnell 2020). Sequencing coverage (Supplementary Figure S1a) was stable throughout the genome at ~14000x, with a marked spike in the region interested by the repeats, suggesting that the length of the repeated sequence may have been underestimated (e.g. Nardi et al. 2024). Automatic annotation was performed using MITOS2 (ver. 2; Bernt et al. 2013) and manually revised in accordance with the *Popillia japonica* (OP974626; Nardi et al. 2024) genome. CR repeats were identified using Tandem Repeats Finder (update 2022; Benson 1999). The genome was prepared for submission using Aln2tbl (Pons et al. 2021) and the genome map was drawn using Chloroplot (Zheng et al. 2020).

All available complete mitochondrial genomes of Rutelinae were downloaded from NCBI (Table 1). Following automatic annotation, *Melolontha melolontha* (Melolonthinae) was included as outgroup. Single PCGs were retroaligned using MUSCLE (ver. 3.8.425; Edgar 2004) in AliView (ver. 1.28; Larsson 2014), end-trimmed and concatenated using EZmito (Cucini et al. 2021). Starting from initial partitions by strand/codon, the evolutionary model was optimized using Partition Finder (Lanfear et al. 2012) and Model Finder (Kalyaanamoorthy et al. 2017) in IQtree2 (ver. 2.0.7; Minh et al. 2020). The Maximum Likelihood tree, including

Table 1. Species included in the phylogenetic analysis.

Species	NCBI	Reference	Sub-family	Tribe	Sub-tribe
Popillia japonica	OP974626	Nardi et al. 2024	Rutelinae	Anomalini	Popillina
Popillia mutans	MF997049	Song and Zhang 2018	Rutelinae	Anomalini	Popillina
Anomala aulax	PP350013	unpublished	Rutelinae	Anomalini	Anomalina
Anomala corpulenta	OL449520	Qu et al. 2023	Rutelinae	Anomalini	Anomalina
Anomala rufiventris	OR208200	Long et al. 2024	Rutelinae	Anomalini	Anomalina
Anomala russiventris	MW829593	Li et al. 2022	Rutelinae	Anomalini	Anomalina
Mimela junii	PQ067309	This study	Rutelinae	Anomalini	Anomalina
*Mimela splendens**	MZ064554	unpublished	Rutelinae	Anomalini	Anomalina
Callistethus plagiicollis	OR208201	Long et al. 2024	Rutelinae	Anomalini	Anomalina
*Adoretus sp.***	JX412788	Timmermans et al. 2015	Rutelinae	Adoretini	Adoretina
Melolontha melolontha	OW285245	Ashworth 2023	Melolonthinae	Melolonthini	Melolonthina

*species attribution tentatively not confirmed. **incomplete genome including compete PCGs.

bootstrap support, was identified in IQtree2. The analysis was repeated based on 1st and 2nd codon positions as well as on amino-acid sequences. The optimal Bayesian tree, with posterior probabilities, was identified in BEAST2 (ver. 2.7.7; Bouckaert et al. 2014) applying the same partitions and model.

Results

The mitochondrial genome of *M. junii* is a circular molecule 16,805 bp long and conforms, in structure and gene content, to the model generally observed in Metazoa (Figure 2). It encodes for the canonical 13 protein subunits (*cox1/2/3*, *atp8/6*, *nad2/3/6*, *cob* on the *plus* strand; *nad1/4/4L/5* on the *minus* strand), two ribosomal RNAs (*rrnS*, *rrnL*, on the *minus* strand), a complete set of 22 tRNAs (*trnI, trnM, trnW, trnL^{uur}, trnK, trnD, trnG, trnA, trnR, trnN, trnS^{agn}, trnE, trnT, trnS^{ucn}* on the *plus* strand; *trnQ, trnC, trnY, trnF, trnH, trnP, trnL^{cun}, trnV* on the *minus* strand) as well as a long non coding region between *rrnS* and *trnI* (CR: 2114 bp). An array of repeats is observed in the CR (Supplementary Figure S1b). The genome organization is compact, with 11 gene overlaps (1 to 8 bp, total 40 bp) and six small spacers (1 to 20 bp, total 42 bp). All PCGs start with a canonical initiation codon. Seven PCGs end with a canonical termination codon and five with an incomplete T signal. The gene order conforms to the Pancrustacea model.

All phylogenetic analyses converged to the same topology (Figure 3), apart from a weakly supported node in the amino-acid dataset. *M. junii* appears to be nested within genus *Anomala* with high support and related to *A. corpulenta,* as well as the unconfirmed *M. splendens,* with medium/low support.

Discussion and conclusion

The uncertainty in determining the number of CR repeats may be due to a methodological bias, as Illumina short reads are not appropriate to resolve complex repeated structures

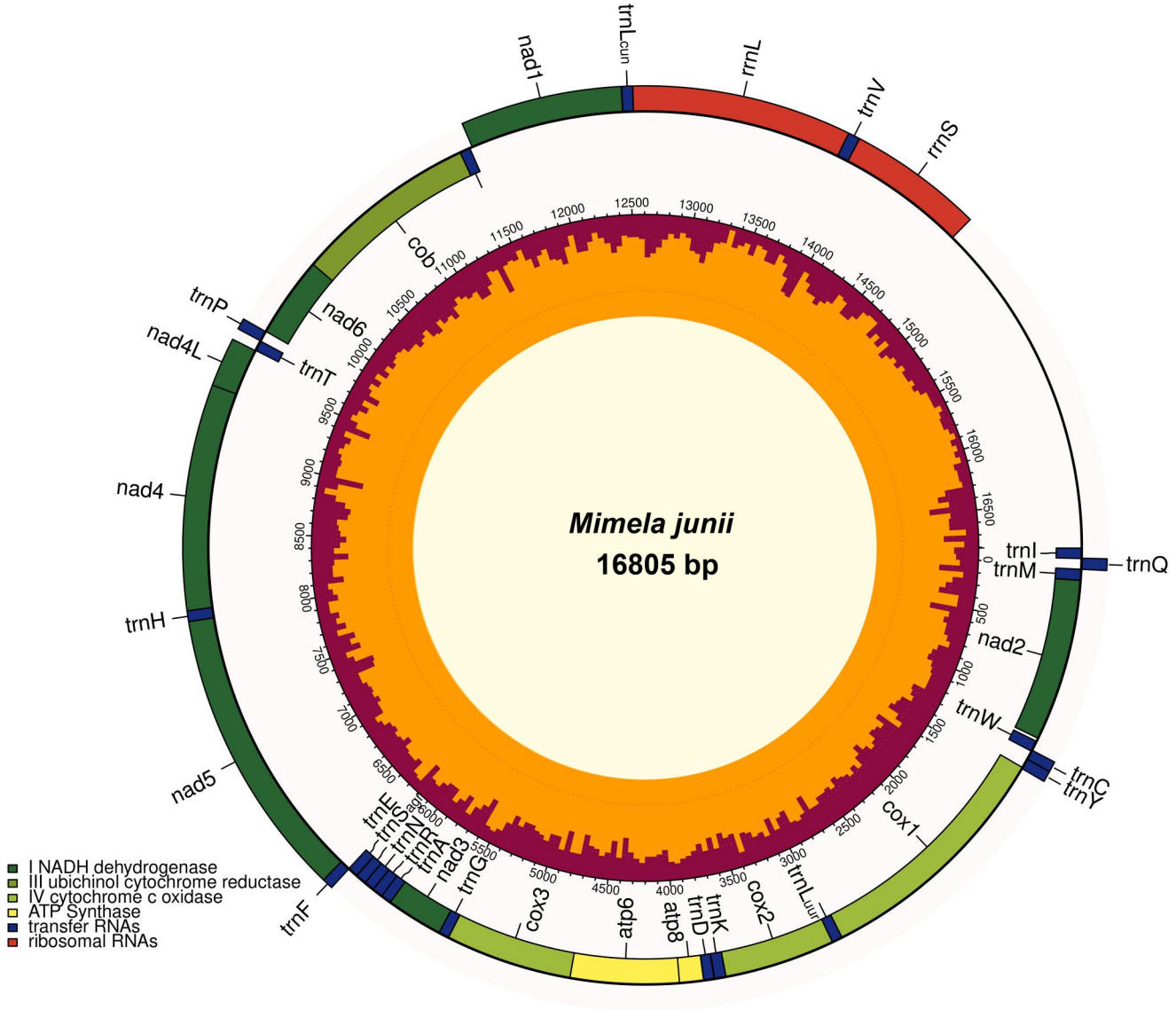

Figure 2. *Mimela junii* mitochondrial genome map. Genes encoded on the *plus* strand are indicated in the inner circle, genes encoded in the *minus* strand are indicated in the outer circle. GC content is shown within the map.

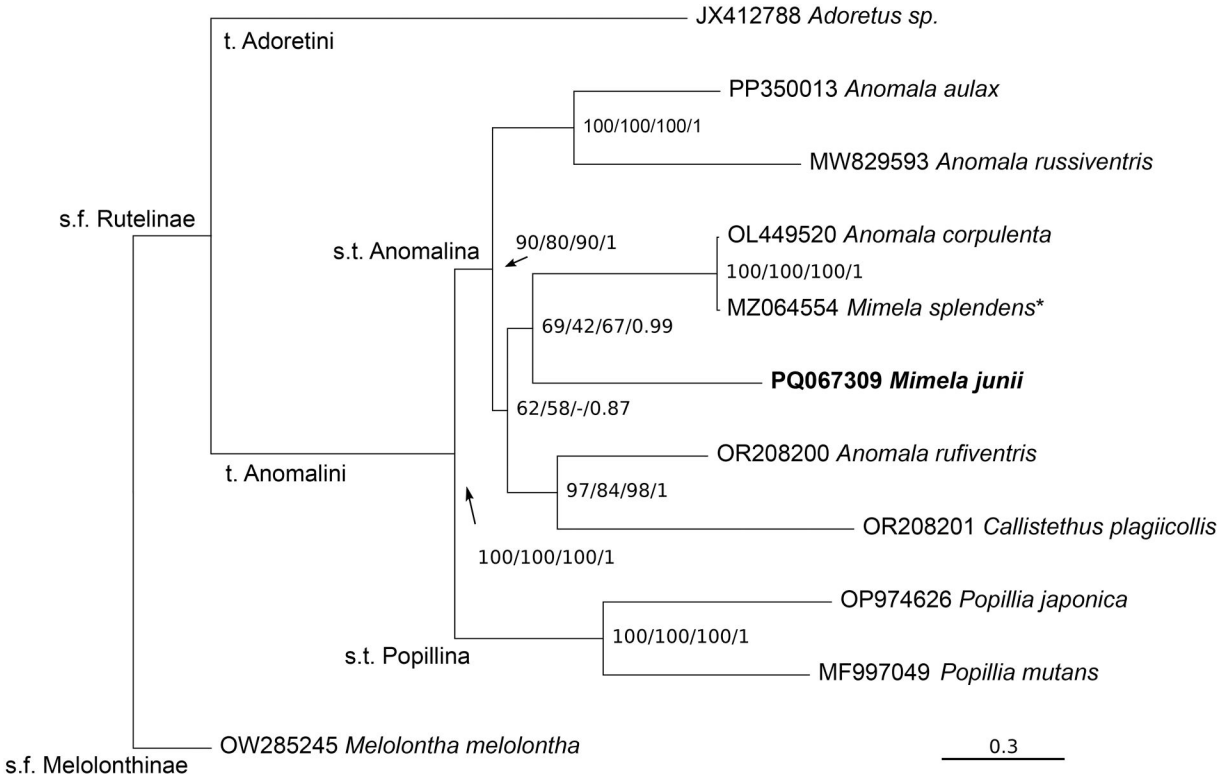

Figure 3. Phylogenetic tree of *Mimela junii* (in bold) in the context of Sub-family Rutelinae. Nodes corresponding to named taxa are thus labeled. s.f. sub-family, t. tribe, s.t. sub-tribe. Branch lengths are from the 1+2+3 codon position analysis; root node not to scale. Support at nodes is indicated as: boostrap 1+2+3 codon position/bootstrap 1+2 codon position/bootstrap aminoacids/posterior probability 1+2+3 codon position. *species attribution tentatively not confirmed. See Table 1 for full species information.

(e.g. Nardi et al. 2024), or have a biological basis, as heteroplasmy in the number of repeats (Nardi et al. 2001, 2012) may be at play. This suggest caution in the interpretation of CR assemblies obtained using short reads and underlines the relevance of the few complete mitochondrial genomes produced based on a combination of long and short reads (e.g. Gastineau et al. 2024; Nardi et al. 2024).

Sequence MZ064554, identified in NCBI as *Mimela splendens*, was tentatively considered as of unconfirmed attribution because: a) it is almost identical (99.4%) to sequence OL449520 (identified as *A. corpulenta* in NCBI); and b) both are identified as *A. corpulenta* based on their barcode in BOLD.

Based on our phylogenetic reconstruction (Figure 3), and in line with the accepted classification of the group (Bouchard et al. 2011), sub-family (s.f.) Rutelinae includes tribe (t.) Adoretini and t. Anomalini, and the latter sub-tribe (s.t.) Popillina and s.t. Anomalina. Within Anomalina, the genus *Anomala* is recovered as paraphyletic with respect to *Mimela* and *Callisthetus,* with *M. junii* nested within genus *Anomala* and associated with *A. corpulenta* and (the unconfirmed) *M. splendens*. Mitochondrial genomes of Rutelinae have been included into multiple phylogenetic analyses (Timmermans et al. 2015; Song and Zhang 2018; Li et al. 2022; Qu et al. 2023; Long et al. 2024). Limited to shared sequences, our results are identical to previously published phylogenetic trees.

The availability of the mitochondrial genome of *M. junii* and other Rutelinae is liable to foster molecular identification, including preimaginal stages. Future work along this line should consider sequencing additional mitochondrial genomes from Rutelinae. We nevertheless wish to underline that molecular identification is only as good as the underlying taxonomic identification of the reference material. As such the source material should be confirmed by positive, multi-disciplinary expertise, especially in species-rich and economically relevant taxa such as shining leaf chafers.

The complete sequence of the mitochondrial genome of the shining leaf chafer *Mimela junii* herein described will promote molecular identification of leaf chafers, a group that include relevant pest, alongside many economically irrelevant, species.

Authors contributions statement

FF conceived the study; FF and DB provided samples; FN, CC, RF and AC conducted experiments and analyzed the data; FN drafted the manuscript. All authors reviewed and edited the manuscript.

Ethical statement

The research was exempt from ethical approval or permissions because it does not involve regulated species.

Disclosure statement

No potential competing interest was reported by the authors.

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Data availability statement

The genome sequence is publicly available in Genbank of NCBI (https://www.ncbi.nlm.nih.gov/) under accession number PQ067309. The associated BioProject, SRA, and Bio-Sample codes are PRJNA1139055, SRR29924876, and SAMN42749332, respectively.

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