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MITOGENOME ANNOUNCEMENT

Characterization of the complete mitochondrial genome of a forensically important beetle, *Ptomascopus plagiatus* (Coleoptera: staphylinidae: silphinae)

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ABSTRACT

Ptomascopus plagiatus (Ménétriés, 1854) is a forensically important silphid species. In this study, we report on the mitochondrial genome of *P. plagiatus*. The complete mitochondrial genome of *P. plagiatus* is 17556 bp and contains 22 transfer RNA genes, 13 protein-coding genes (PCGs), two ribosomal RNA genes, and a 2953 bp noncoding region. The nucleotide composition of *P. plagiatus* is biased toward A and T (A + T: 77.46%). Phylogenetic analysis based on mitogenomic data supports that *P. plagiatus* is closely related to (*Nicrophorus nepalensis* Hope, 1831 + *Nicrophorus vespilloides* Herbst, 1783) within the subfamily Silphinae.

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Introduction

Species of subfamily Silphinae are of forensic importance due to their quick colonization of carcasses and relatively long development time (Byrd and Castner 2010; Ridgeway et al. 2014; Montoya-Molina et al. 2021). *Ptomascopus plagiatus* (Ménétriés, 1854) is a widely distributed silphid species in China (Zheng and Gui 1999). Adults and larvae of this species are often collected from buried pork baits at 30 cm depth in Shenyang from late June to late October (Zou et al. 2022). Accurate species identification is the prerequisite for the postmortem interval (PMI) estimation using the necrophagous insect succession pattern and development time in forensic entomology (Ren et al. 2021). In insect research, the mitochondrial genome plays an important role in phylogenetic inference and species identification (Cameron et al. 2007; Shang et al. 2019). However, information on the mitochondrial genome of *P. plagiatus* is lacking, which limits its application in forensic investigations. Therefore, we first provided the complete mitochondrial genome sequence of *P. plagiatus* in this study.

Materials

Adults of *P. plagiatus* (Figure 1) were collected using traps baited with pork in Shenyang, Liaoning province, China (41°48′N, 123°25′E). Specimens were identified following the taxonomic keys (Ji 2012). The specimens and DNA were stored at the Natural History Museum of Shenyang University (https://museum.syu.edu.cn/, Dianxing Feng, fdx0808@163.com) under the voucher number SYU-FD-SIL001.

Methods

Genomic DNA of muscle tissues from legs and thorax was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA was used to construct a library with 350 bp fragments and sequenced using a paired-end strategy 2 × 150 bp on the Novaseq 6000 platform (Illumina, USA) at the commercial corporation (Genepioneer Biotechnologies Co. Ltd., Nanjing, China). The raw reads were filtered using Fastp v0.23.0 (Chen et al. 2018). The putative mitochondrial reads were obtained by mapping reads to the local database (including all the published mitochondrial genomes of Coleoptera downloaded from NCBI GenBank database) using Bowtie2 v2.2.6 (Langdon 2015). The assembler SPAdes v3.14.1 (Bankevich et al. 2012) was used for assembly. To verify the accuracy of the assembly, the coverage depth (3231 × ∼ 7994 ×, mean: 7088.15 ×, Supplementary Figure 1) was computed using Samtools v1.16.1 (Ni et al. 2023). Additional, the assembled genome was also aligned with the mitochondrial genome of *Nicrophorus nepalensis* Hope, 1831 (MW365941) (Cai and Li 2021) using Geneious v11.0.4 (Kearse et al. 2012). The mitochondrial genome was annotated by MITOS2 online server under the invertebrate mitochondrial code (Bernt et al. 2013) and manually verified using the NCBI database. The base composition and

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codon usage were calculated by MEGA v11.0.13 (Tamura et al. 2021). The AT and GC skews were calculated according to the formulae: AT skew = (A − T)/(A + T) and GC skew = (G − C)/(G + C) (Perna and Kocher 1995). The final genome map was plotted by CGView online server (Grant and Stothard 2008).

Because the mitochondrial genomes of some silphid species available in the NCBI GenBank database were incomplete and unverified, only the annotated genomes containing at least 13 PCGs and 2 rRNAs sequences were selected for phylogenetic analysis (Table 1). The mitochondrial genome of *Tetartopeus terminatus* (Gravenhorst, 1802) from the subfamily Paederinae was chosen as an outgroup (Kim et al. 2023). Sequences were aligned by MAFFT v7.511 (Katoh et al. 2002). The GTRGAMMA model was used to construct a ML tree in RAxML v8.2.10 with a bootstrap value of 1000 (Minh et al. 2013).

Figure 1. Adult of *Ptomascopus plagiatus* (Ménétriés, 1854) (photographed by shutong dai). This species has an orange-red rectangle band on each elytra. The dorsal plate of anterior thorax has dense grayish-yellow appressed hairs. The Middle tibiae are straight or slightly curved, and the hind tibiae are straight. Bar = 1 cm.

Results

The complete mitochondrial genome of *P. plagiatus* (GenBank accession no. OP250947) is 17556 bp in length, and contains 22 tRNAs, 13 PCGs, two rRNAs, and one noncoding region which was 2953 bp long (Figure 2). The nucleotide composition of the mitochondrial genome is 40.38% of A, 37.08% of T, 8.66% of G, and 13.88% of C. The proportion of A + T (77.46%) is higher than G + C (22.54%), and AT skew is positive (0.043). Four PCGs (*NAD1*, *NAD4*, *NAD4L*, and *NAD5*), eight tRNAs (*trnQ*, *trnC*, *trnY*, *trnF*, *trnH*, t*rnP*, *trnL1*, *trnV*) and two rRNAs *(rrnS*, *rrnL)* are encoded by the light strand, while others are located on the heavy strand (Figure 2.). Among the 13 PCGs, six genes (*COX1*, *ATP8*, *NAD2*, *NAD3*, *NAD5* and *NAD6*) begin with ATT codon, five genes (*ATP6*, *NAD4*, *NAD4L*, *COX3,* and *COB*) begin with ATG codon, and two genes (*COX2* and *NAD1*) start with ATA codon. Five genes (*ATP6*, *ATP8*, *NAD2*, *NAD4L,* and *NAD6*) end with TAA codon, and the rest of the genes except *NAD1* (TAG codon) stop with T, the incomplete stop codon. The 13 PCGs encode 3662 codons (excluding stop codons). The four most frequently used codons are UUA, AUU, UUU and AUA, which are used 448, 342, 296, and 230 times, respectively.

The phylogenetic analysis is conducted using 13 PCGs and 2 rRNAs sequences of eight silphid species and one outgroup species. The ML tree shows the eight silphid species cluster together and form the subfamily Silphinae (Figure 3). There are two groups in this subfamily. In the first group, species from the genera *Necrodes*, *Diamesus*, *Oiceoptoma* and *Necrophila* are grouped together to form the tribe Silphini. In the other group, *P. plagiatus* is closely related to the species of genus *Nicrophorus*, which together constitute the tribe Nicrophorini.

Discussion and conclusion

The circular mitochondrial genome of *P. plagiatus* is 17556 bp long. Like other typical insect mitochondrial genomes, it contains 13 PCGs, 22 tRNAs, 2 rRNAs, and a noncoding region. The nucleotide composition has obvious A-T bias.

Table 1. List of the mitochondrial genomes used in phylogenetic analysis.

Subfamily	Species	GenBank accession no.	Mitochondrial genome size (bp)	References
Silphinae	*Ptomascopus plagiatus* (Ménétriés, 1854)	OP250947	17556	This study
	Nicrophorus vespilloides (Herbst, 1783)	MT872689	15292	Unpublished
	Nicrophorus nepalensis (Hope, 1831)	MW365941	17299	Cai and Li (2021)
	Necrodes nigricornis (Harold, 1875)	OP712657	18503	Kim et al. (2023)
	Necrodes littoralis (Linnaeus, 1758)	MW415274	17830	Jiang et al. (2021)
	Diamesus osculans (Vigors, 1825)	MN900864	19400	Zhang et al. (2020)
	Oiceoptoma thoracicum (Linnaeus, 1758)	OL397054	18540	Unpublished
	Necrophila americana (Linnaeus, 1759)	GU176343	16902	Song et al. (2010)
Paederinae	*Tetartopeus terminatus* (Gravenhorst, 1802)	KT780698	17652	Unpublished

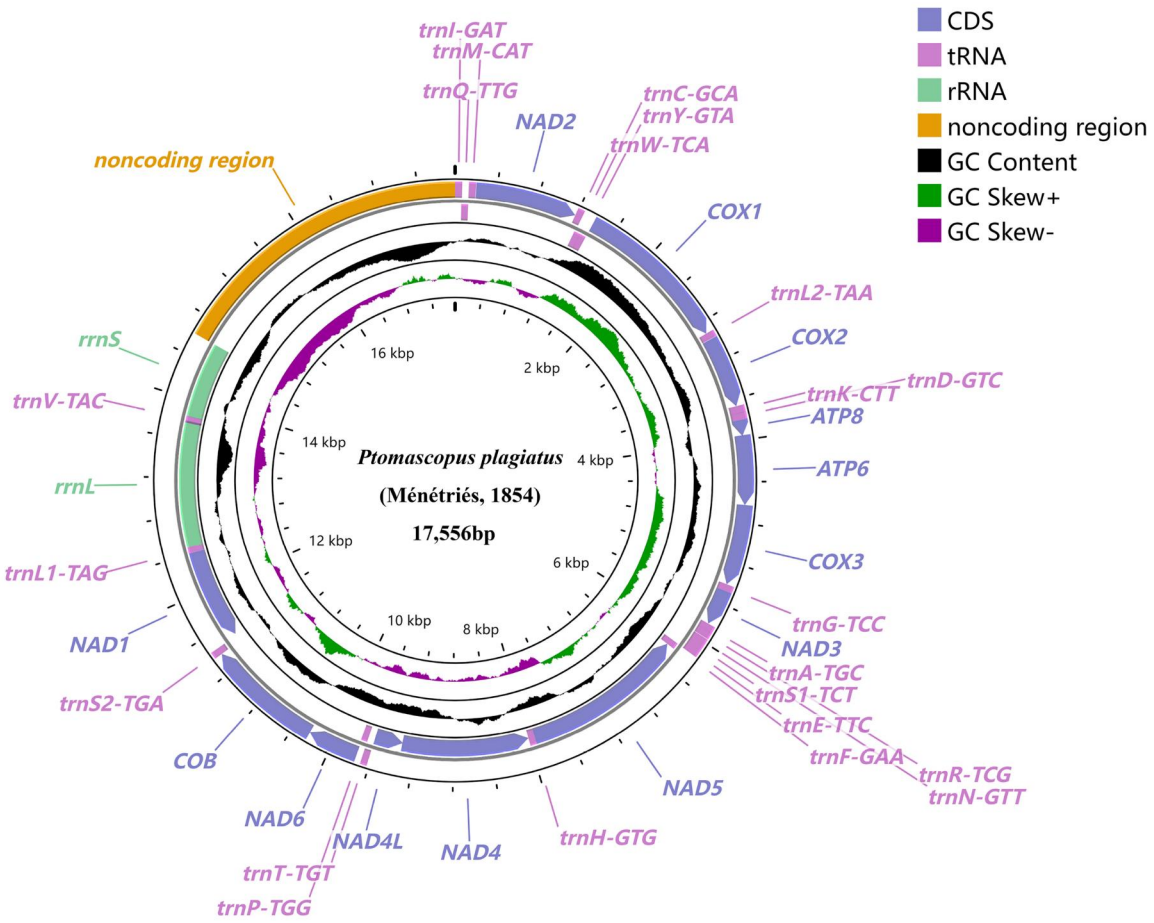

Figure 2. Mitochondrial genome map of *Ptomascopus plagiatus* (Ménétriés, 1854) displaying both heavy (outside the circle) and light (inside the circle) strands. The transcriptional directions of genes located on the heavy and light strands are clockwise and counterclockwise, respectively. Plots of GC content and skew used a sliding window size of 500 and step size of 1. GC skew is plotted using green and purple sliding windows as the deviation from the average of the mitochondrial genome.

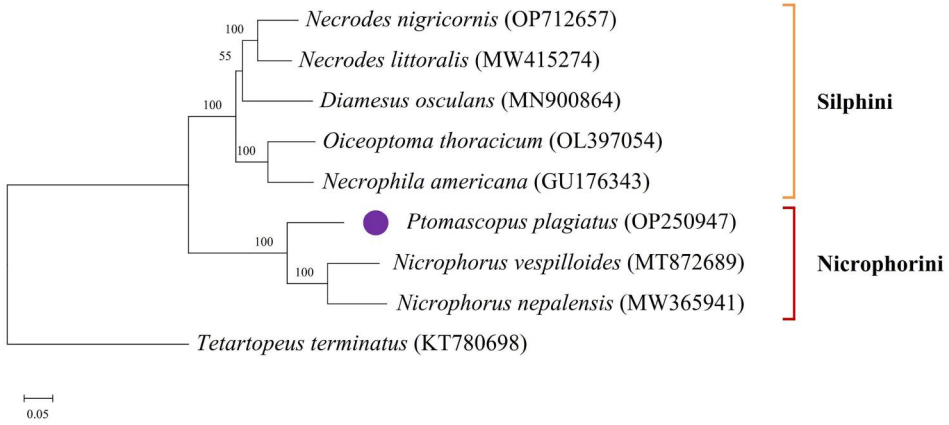

Figure 3. ML tree was constructed using mitochondrial genome sequences of 8 species from the Silphinae and an outgroup species using RAxML v8.2.10 software. Numbers at nodes indicate bootstrap values. GenBank accession numbers are given adjacent to the species name.

Traditionally, Silphidae was a separate family that included two subfamilies, Nicrophorinae and Silphinae. Recently this family was formally downgraded to a subfamily of Staphylinidae (Cai et al. 2022; Gruszka and Matuszewski 2023; Růžička et al. 2023). Correspondingly, Nicrophorinae and Silphinae were relegated to two tribes, Nicrophorini and Silphini, respectively. The molecular phylogenetic analysis supports that *P. plagiatus* belongs to the tribe Nicrophorini and is close to (*N. vespilloides* + *N. nepalensis*) within the subfamily Silphinae. The annotated mitochondrial genome of *P. plagiatus* provided by this study is extremely valuable for the species identification and phylogenetic analysis of the subfamily Silphinae.

Ethical approval

Ptomascopus plagiatus (Ménétriés, 1854) is a saprophagous species easily trapped using pork baits. The samples collected and used for this study

do not involve humans or animals. Therefore, this study does not need ethical approval or permissions to collect the samples.

Authors contributions

WH performed the experiments, analyzed the data and wrote the original manuscript. DF was involved in the conception, design and revising the manuscript. SD collected and photographed the specimen, and analyzed the data. All authors have approved the manuscript for publication and agreed to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest is reported by the authors.

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Data availability statement

Mitogenome data supporting this study are openly available in GenBank at nucleotide database, https://www.ncbi.nlm.nih.gov/nuccore/OP250947.1. The associated BioProject, SRA, and BioSample numbers are PRJNA876787, SRR21634603, and SAMN30815897, respectively.

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