The complete mitochondria genome of *Aldrichina grahami* (Diptera: Calliphoridae)

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**ABSTRACT**

*Aldrichina grahami* is a significant medical and forensic insect belonging to the family calliphoridae. In this study, we present the complete mitochondrial genome of *A. grahami* for the first time. The mitogenome was 14903 bp in length, consisting of 22 transfer RNA genes, 2 ribosomal RNA genes and 13 protein-encoding genes. The overall base compositions of A, G, C and T were 39.14, 9.59, 13.65 and 37.62\%, respectively. Phylogenetic analysis is conducted using 12 mitochondrial genomes from two families. The monophyletic branches of the phylogenetic tree reveal that the composition of the mitochondrial genome, particularly the gene lengths, is very similar to that of another Calliphoridae fly, *C. vicina*, but the interspecific variations between them was 5.4\%, which could distinguish these two species explicitly. Phylogenetic analyses indicated that phylogenetic analysis of whole mt genome sequences resulted in much stronger support for the identification of *A. grahami* between other species. This article aimed to enrich the dipteran mitochondrial genomes and provides the complete mitochondrial genome and phylogenetic analyses of *A. grahami* for species identifications forensic purpose.

Accurate postmortem interval (PMI) estimation has been a focus and a difficulty in forensic practices, especially in putrefactive cases (Yan et al. 2014a). *Aldrichina grahami* (Aldrich, 1930), which belongs to the Aldrichina Townsend genus, Calliphoridae family and Diptera order, is widespread in China except Xinjiang Uygur Autonomous Region. *A. grahami* is the first wave of sarcosaphagous flies to arrive and oviposit into animal carcasses, and it has been an important essential in forensic entomology (Xu et al. 2014). Compared with published growth data from larva of the same species, investigators can approximately infer the age of the larva and estimate the PMI (Guo et al. 2011). Viewing it as a potential marker in forensic science, we report the complete mitochondrial genome sequence of *A. grahami* for species identification and phylogenetic analysis (GenBank accession number: KP872701).

The *A. grahami* samples were collected in June 2014 from Changsha city and cultured in the laboratory of Central South University. The entire genome was amplified in eight fragments (Nelson et al. 2012a, 2012b). All of the fragments were amplified by TaKaRa MightyAmp Taq (Takara, Dalian, China) and performed on an Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany). PCR amplifications were performed in 20\(\mu\)L reaction volumes containing 0.5\(\mu\)L of DNA, 0.5\(\mu\)L of each primer, 1.0\(\mu\)L (1.25 U) of TaKaRa MightyAmp Taq polymerase, 10\(\mu\)L of MightyAmp PCR Buffer and 7.5\(\mu\)L of sterilized distilled water. The PCR thermal cycling conditions were as follows: denaturation for 2 min at 94\(^\circ\)C, followed by 35 cycles at 98\(^\circ\)C for 10 s, 50\(^\circ\)C for 30 s, 68\(^\circ\)C for 2.5 min and a final elongation at 68\(^\circ\)C for 10 min. DNA fragments were sequenced on both strands using Sanger dyeoxy sequencing method by the commercial service (Transduction Bio Co. Ltd. Wuhan, China). The complete genome of *A. grahami* is circular 14903 bp in length with 22 transfer RNA genes, 13 protein-coding genes, 2 ribosomal RNA genes and a control region as in other insects. The 89 bp misc_feature region of *A. grahami* was located between 12S rRNA and tRNA-Ile. The genome is particularly AT-biased, with 76.76\% of the nucleotides being either A or T, which is slightly more AT-rich than that for *Parasarcophega similis* (76.37\%) (Yan et al. 2014b) and *Sarcophaga afericia* (75.74\%) (Fu et al. 2014), but less AT-rich than that for *Ravinia perinix* (77.17\%) (Guo et al. 2014). Among the 13 protein-coding genes, 12 were identified with ATN as start codon coding for methionine except COI.

Phylogenetic analysis is conducted using the 12 complete mitochondria gene sequences from six species of Calliphoridae, along with two from family Sarcophagidae as outgroup species. Two of the Calliphoridae species, *L. cuprina* and *L. sericata*, include multiple specimens, allowing genomic variability within these species to be assessed. The sequence data from GenBank which are *A. grahami*, *C. vicina*, *C. rufilaces*, *P. terraenovae*, four *L. cuprina*, two *L. sericata* of Calliphoridae and *S. crassipalpis*, *S. similis* from family Sarcophagidae. Except *A. grahami* and *S. crassipalpis*, the other Calliphoridae genomes originally published in Nelson LA’s paper (Nelson et al. 2012b), *S. similis* published in Yan’s (Yan et al. 2014b) and *S. crassipalpis* published in Ramakodi MP’s (Ramakodi et al. 2015). Each of the 12 mt genomes was...
aligned separately using Vector NTI 9.0. (Lu & Moriyama 2004) and individually aligned gene data sets were translated into amino acid sequences using MEGA 5 (Tamura et al. 2011). The evolutionary history was deduced using the neighbor-joining method (Saitou & Nei 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) was conducted to represent the evolutionary history of the analyzed taxa (Felsenstein, 1985). Branches corresponding to partitions reproduced in <50% bootstrap replicates were collapsed. The percentage of replicate trees is shown next to the branches, in which the associated taxa clustered together in the bootstrap test (500 replicates). The maximum composite likelihood method was used to compute the evolutionary distances (Tamura et al. 2004). Codon positions included were first + second + third non-coding.

The phylogeny of Calliphoridae flies based on the complete mitochondria gene sequences was separated into several genetic clades (Figure 1). As outgroup, the two Sarcophagidae samples were clustered together and clearly separated from the Calliphoridaid mitotypes. The monophyletic branches of the phylogenetic tree reveal that the composition of the A. grahami mitochondrial genome, particularly the gene lengths, is very similar to that of another Calliphoridae fly, C. vicina, but the interspecific variations between them was 5.4%, which could distinguish these two species explicitly. The interspecific and intraspecific percentage genetic divergences were calculated. All values for maximum intraspecific variations of the Calliphoridae species were no more than 2%. The interspecific variations between species were larger than 5% except for L. cuprina and L. sericata, which were no more than 2%, the results is consistent with the previous study (Nelson et al. 2012a, 2012b), which indicated that phylogenetic analysis of whole mt genome sequences resulted in much stronger support for discrimination between A. grahami and other five species, but week for discrimination between the L. cuprina and L. sericata.

Species identification of sarcosaphagous flies using morphological methods is a difficult task, especially for spawn and larva. Forensic scientists have to wait for the adult emergence (Wang et al. 2002). Now species identification techniques of molecular biology including molecular taxonomy serve as an effective supplement for morphological identification. We hope the complete mitochondrial genome and phylogenetic analysis of A. grahami can be valuable for species identification and will be instrumental for implementation of the Calliphoridae database.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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