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MITOGENOME ANNOUNCEMENT

First report of the mitogenome of *Hamaxiella brunnescens* (Diptera, Tachinidae) from Beijing, China

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ABSTRACT

Hamaxiella brunnescens (Mesnil, 1967) (Diptera, Tachinidae) is a parasitic fly species and of great ecological importance in natural systems as parasitoids of herbivorous insects. The mitogenome of *H. brunnescens* was sequenced and analyzed here for the first time. The genome is 14,956 bp in length with high A + T content, which consists of 13 protein-coding, 22 tRNA, two rRNA genes, and a partial non-coding control region. The phylogenetic analyses support a monophyletic Tachinidae. The two subfamilies Exoristinae and Phasiinae are fully supported as monophyletic while Tachininae is inferred to be paraphyletic.

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Hamaxiella brunnescens (Mesnil, 1967) is classified in the Tachininae of the family Tachinidae, which is one of the most diverse group of parasitoid Diptera with around 10,000 described species worldwide (Pape et al. 2011; O'Hara 2012). Many species in the family serve as important natural enemies of herbivorous insects in both natural and managed terrestrial ecosystems, especially forests (Stireman and Singer 2003; Stireman et al. 2006; O'Hara et al. 2009; O'Hara and Cerretti 2016). Due to their variable morphological features, entomologists face great challenges in identifying tachinid flies, and the phylogenetic relationships within the Tachinidae are unclear at the higher level (Meier et al. 2006; O'Hara 2013). Mitogenomic data have been broadly used in molecular identification and phylogenetic studies of Diptera, e.g. Yan et al. (2019) and Li et al. (2020); however, only a few complete Tachinidae mitogenomes have been published. In this study, we analyzed the mitochondrial genome of *H. brunnescens*, as representative of the subfamily Tachininae to document its genome structure, evolutionary history, and to contribute to future genetic research in the family.

The *H. brunnescens* adults were collected from Baihuashan National Nature Reserve of Beijing, China (39.836403N, 115.578186E) in June 2017. Whole genomic DNA was extracted from the muscle tissues of the thorax of an adult using the DNeasy Blood and Tissue kit (QIAGEN Sciences, Valencia, CA). The voucher specimen of *H. brunnescens* was deposited in the Museum of Beijing Forestry University (http://bjfc.bjfu.edu.cn, Wenya Pei, peiwenya@bjfu.edu.cn) under the voucher number BFU14C6-1, and DNA sample was deposited at the Laboratory of Animal Noninvasive Studies on School of Ecology and Nature Conservation, Beijing Forestry University, Beijing, China. The COI was amplified with primer pair LCO1490-L (5′-GGTCWACWAATCATAA AGATATTGG-3′) and HCO2198-L (5′-RAAACTTCWGGRTGWCC AAARAATCA-3′) (Folmer et al. 1994; Nelson et al. 2007) and sequenced using Sanger sequencing (GenBank accession: MW370351), to be used as bait to iterate the mitogenome of *H. brunnescens*. The genomic DNA was subsequently pooled with other dipteran species from other families and sequenced using the Illumina NovaSeq 6000 (PE150, Illumina, San Diego, CA) platform. The software IDBA-1.1.1 (Peng et al. 2012) was employed to assemble the data with the similarity set to be 0.98. The mitogenome of *H. brunnescens* was deciphered using a Blast search (Altschul et al. 1990) with COI as the bait sequence (Crampton-Platt et al. 2015). The mitogenome annotation was completed following Zhang et al. (2016).

The DNA assembly was 14,956 bp in length (GenBank accession no. MW256712), containing 13 protein-coding genes (PCGs), two ribosomal RNA, 22 transfer RNA, and a partial non-coding control region. The overall nucleotide composition was 38.5% of A, 40.5% of T, 8.7% of C, 12.3% of G, and 79% of A + T content. Most of the 13 PCGs used ATN as the start codon (ATG for COII, ATP6, COIII, ND4, ND4L, CYTB, and ND1; ATT for ND2 and ND5; ATA for ND3; ATC for ATP8 and ND6), except that COI begins with codon TCG. The stop codon TAA is assigned to most of the PCGs (ND2, COI, ATP8, ATP6, COIII, ND4L, ND6, and ND1), but an incomplete stop codon T– is used by three PCGs (COII, ND5, and ND4), ND3 and CYTB terminate with the codon TAG.

Mitochondrial genomes of Tachinidae species available from GenBank were mined for the phylogenetic analysis. The

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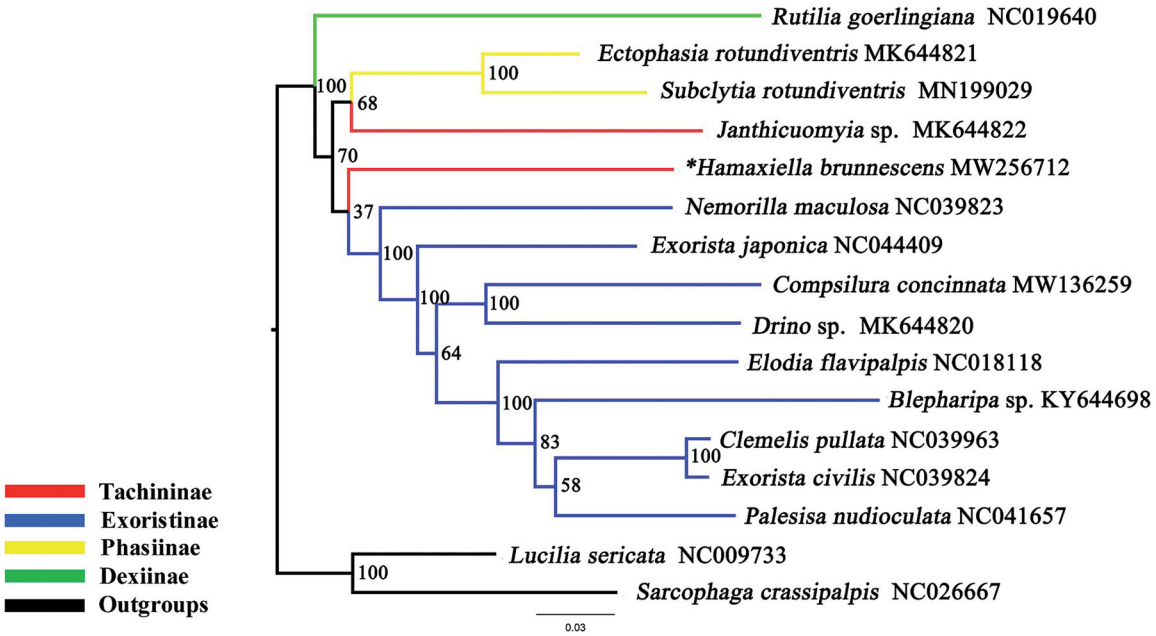

Figure 1. Phylogenetic tree of 16 calyptrate species based on the concatenated dataset of 13 PCGs and two rRNA using the maximum-likelihood (ML) method, with bootstrap values beside nodes based on 100 replicates. The alphanumeric terms following species names indicate the GenBank accession numbers. *Species documented in this study.

maximum-likelihood (ML) reconstruction was performed using IQ-TREE (Nguyen et al. 2015) based on a matrix concatenated with the alignments of 13 PCGs and two rRNAs that was aligned by MAFFT (Katoh and Standley 2013) (Figure 1). The evolutionary models were selected with the 'Auto' option, and a 100 replicate bootstrapping was performed using the 'Non-parametric (complete b)' function implemented in IQ-TREE. *Lucilia sericata* (Calliphoridae) and *Sarcophaga crassipalpis* (Sarcophagidae) were designated as outgroups. The result clearly shows that the Tachinidae formed a monophyletic group with full support (Figure 1). The subfamily Tachininae are inferred as paraphyletic, with the species *H. brunnescens* occupying an unsupported sister position to the Exoristinae, and the species *Janthicuomyia* sp. as a probable sister group to Phasiinae. The monophyly of the remaining subfamilies are confirmed, with the exception of the Dexiinae, which is represented by only one species. These subfamilies level relationships inferred here using mitogenomic data are inconsistent with Stireman et al. (2019), which used nuclear genes for phylogeny reconstruction. The limited taxon sampling here could be one of the reasons for this inconsistence. Another possible reason is the different phylogenetic information contained by mitochondrial and nuclear genes, as suggested by Zhang et al. (2016), Kutty et al. (2019), and Yan et al. (2020).

Disclosure statement

No potential competing interest was reported by the author(s).

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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/MW256712, Associated BioProject, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA681393, BioSample accession number at https://www.ncbi.nlm.nih.gov/biosample/SAMN16952152, and Sequence Read Archive at https://www.ncbi.nlm.nih.gov/sra/SRP295347.

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