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MITOGENOME ANNOUNCEMENT

Complete mitogenome sequence of *Aedes (Hulecoeteomyia) japonicus japonicus* from Hawai'i Island

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ABSTRACT

We report the first complete mitogenome (Mt) sequence of *Aedes japonicus japonicus* (Diptera: Culicidae). The sequence was extracted from one adult from the Big Island of Hawai'i Island. The length of the *Ae. japonicus japonicus* Mt was 16,528bp with 78.1% AT content. Its sequence is most similar to the Mt sequence of *Aedes koreicus* with 90.81% sequence identity. This is the first full Mt sequence available for this species and provides important genetic resource for studying population genetics and dynamics of this important invasive mosquito species.

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Aedes japonicus japonicus; arbovirus vector; Pacific Island; Culicidae; invasive species

Introduction

Aedes (Hulecoeteomyia) japonicus (Theobald 1901), commonly known as the Asian bush mosquito (Figure 1(A,B)), was first documented in Japan in 1901 as *Culex japonicus* (Theobald 1901). *Aedes j. japonicus* has been described as a subspecies of *Ae. japonicus* and can be challenging to morphologically distinguish from other subspecies within *Ae. japonicus* (Tanaka et al. 1979). On the other hand, four subspecies are genetically quite distinct from each other (Cameron et al. 2010). Based on the re-examination of morphological characteristics, evidence of molecular divergence, and apparent allopatric distributions of the subspecies of *Ae. japonicus*, Wilkerson et al. suggested elevating *Ae. j. japonicus* and other subspecies to species rank recently (Wilkerson et al. 2022).

Aedes j. japonicus is more common than other subspecies, and its native distribution area is Palearctic region while other subspecies is found in Oriental region of Japan (Tanaka et al. 1979; Cameron et al. 2010). Interaction of *Ae. j. japonicus* with international commerce has been implicated in its invasion and spread in North America and Europe (Cameron et al. 2010). Its slow expansion in the United States of America could be explained by its limited tolerance for higher ambient temperatures in coastal habitats (Kaufman and Fonseca 2014), although this species has been established in subtropical Florida (Riles et al. 2017) and the tropical Hawaiian Islands (Larish and Savage 2005; Larish et al. 2010; Matsunaga et al. 2019).

Ecological impacts of its expansion are recorded in its interspecific associations with other container-inhabiting mosquitoes including disease vector (Armistead et al. 2008; Andreadis and Wolfe 2010), and therefore may indirectly affect the local disease dynamics. In North America, *Aedes j. japonicus* has been found naturally infected with several pathogens (Harris et al. 2015; Silaghi et al. 2017; Yang et al. 2018; DeCarlo et al. 2020) including Cache Valley virus, Heartworm, La Crosse Virus, and West Nile virus. However, its role in transmission can be considered ambiguous and requires further investigations. In vector competence experiments, *Ae. j. japonicus* has been determined to be an efficient laboratory vector due to its susceptibility to various arboviruses crossing salivary gland barriers (Turell et al. 2013; Abbo et al. 2020).

Materials and methods

The adult *Aedes japonicus* specimen was collected from Hawai'i (19.0853°N, 155.7757°W) using a BG-sentinel mosquito trap (Biogents, Regensburg, Germany) baited with BG-Sweetscent Attractant. DNA extraction and library preparations were conducted as previously described (Chen et al. 2021; Kelly et al. 2021). Extracted DNA sample (voucher accession number: Ae21OCNV006, contact person: Yoosook Lee, yoosook.lee@ufl.edu) was kept in the Florida Medical Entomology Laboratory at the University of Florida.

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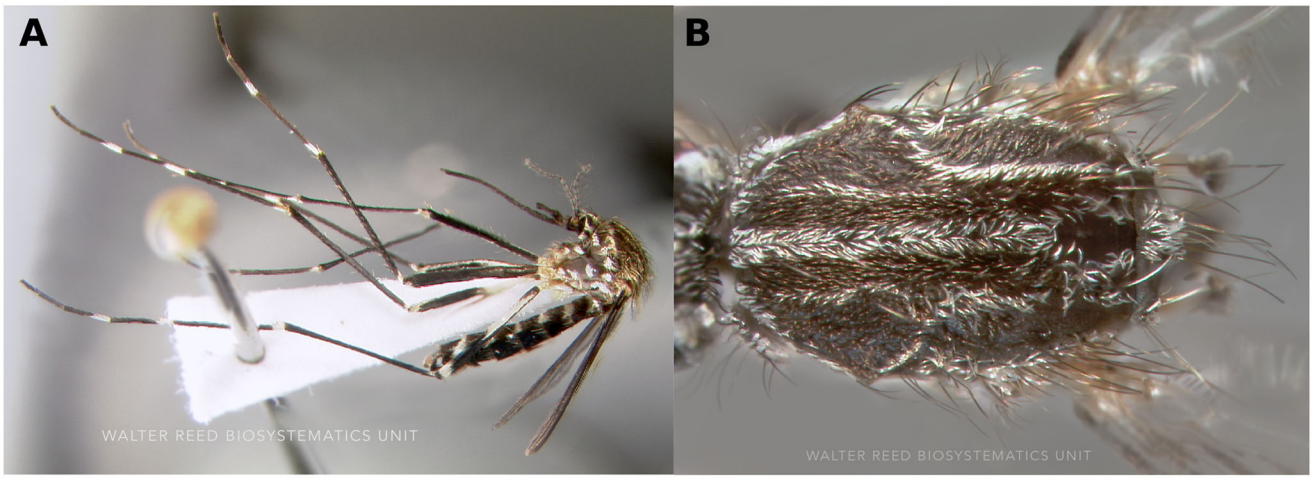

Figure 1. *Aedes japonicus* reference image. Courtesy of David Pecor at the Walter Reed Biosystematics Unit (WRBU) (Matsunaga et al. 2019). (A) Habitus in lateral view. (B) Thorax in dorsal view. The scutum is characterized by golden stripes with distinctive lyre-shaped strips, two sub-median and a median strip, which is one of the features used for species identification.

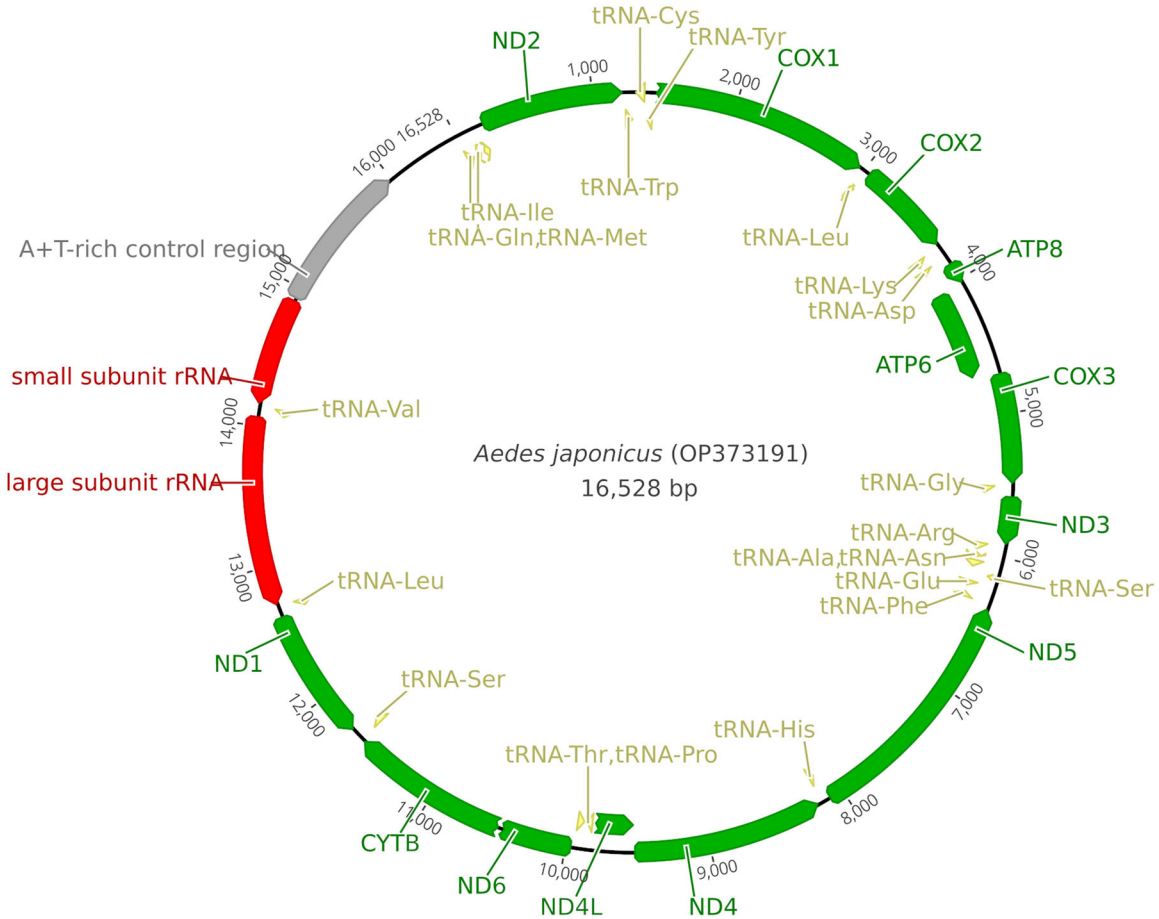

Figure 2. Mitogenome map of *Ae. j. japonicus*. Green ribbons indicate genes; yellow ribbons indicate tRNA-encoding regions; red regions indicate rRNA-encoding regions; and gray ribbon indicates an AT-rich region. The ribbon orientation depicts the direction of transcription.

The library was sequenced for 150 bp paired-end reads using a NovaSeq 6000 instrument (Illumina, San Diego, CA) at the University of Florida Interdisciplinary Center for Biotechnology Research (UF ICBR) NextGen DNA Sequencing Core. Raw sequencing reads were trimmed using fastp version 0.20.1 (Chen et al. 2018). Mt contigs were assembled using NOVOPlasty version 4.2 (Dierckxsens et al. 2017). Following the methods used for *Ae. busckii* and *Ae. taeniorhynchus* (Cornel et al. 2020), automatic annotation of mitogenome (Mt) was conducted with the MITOS website (Bernt et al. 2013) under default settings and the invertebrate genetic code for mitochondria. The Jukes-Cantor model was

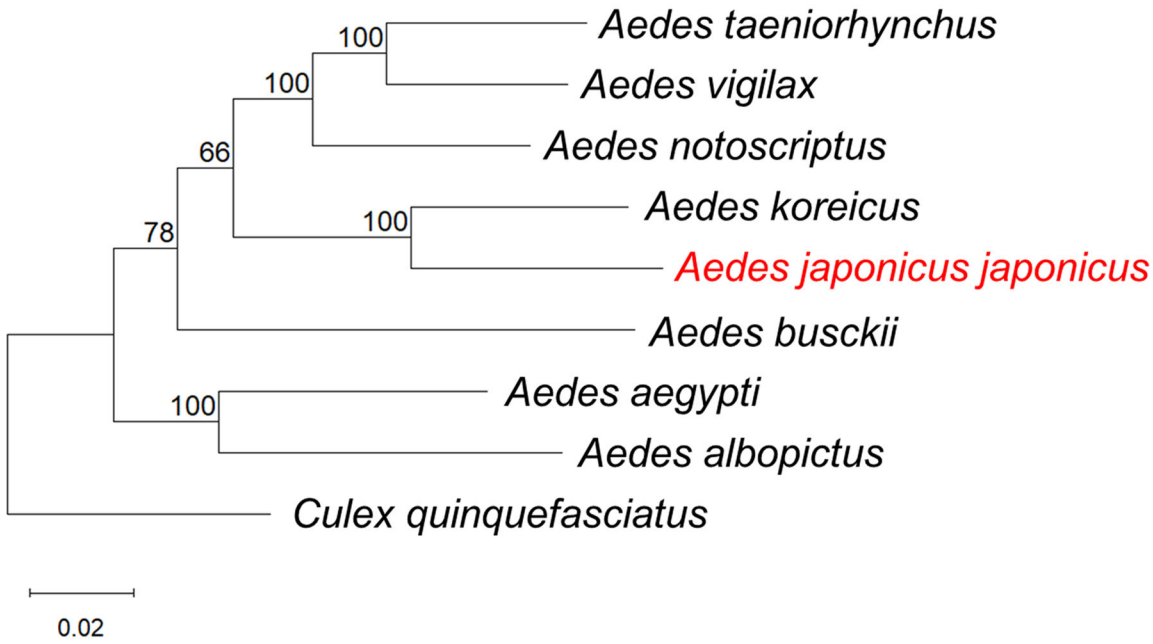

Figure 3. Phylogenetic tree constructed using maximum-likelihood (ML) based on complete mitogenome sequences of related mosquitoes. The following sequences were used: *Aedes taeniorhynchus* MN626442 (Cornel et al. 2020), *Ae. vigilax* KP995260 (Hardy et al. 2016), *Ae. notoscriptus* KM676219 (Hardy et al. 2016), *Ae. koreicus* MT093832 (Shin and Jung 2020), *Ae. japonicus japonicus* OP373191 (this study), *Ae. busckii* MN626443 (Cornel et al. 2020), *Ae. aegypti* MH348176 (Schmidt et al. 2018), *Ae. albopictus* NC_006817 (Ho et al. 2021), *Culex quinquefasciatus* HQ724617 (Atyame et al. 2016). Numbers at nodes indicate bootstrap frequencies out of 500 replicates. *Culex quinquefasciatus* was considered as an outgroup. The scale bar indicates relative nucleotide difference (0.02 = 2% nucleotide difference).

used to build the phylogenetic tree with the maximum likelihood method using MEGA11 software version 11.0.13 (Kearse et al. 2012).

Results

Our NovoPlasty run yielded a single circular DNA contig of *Ae. j. japonicus* mitochondrion. The average organelle coverage was 3298X. The mitochondrial genome was 0.55% of the extracted mosquito genomic DNA. The length of the *Ae. j. japonicus* Mt (GenBank accession number: OP373191) was 16,528 bp and the percentage of A + T was 78.1% (Figure 2). The cytochrome c oxidase I (COI) fragment spanning 1458–2166 bp of *Ae. j. japonicus* sequence was 99.49% (±0.2 SD) identical to the COI sequences of *Ae. j. japonicus* deposited in GenBank. Some of automatic annotations did not end with stop codon. In such cases, we revised the end points manually to properly align with stop codons. Annotation information was also deposited to the GenBank with the genome sequence. Genomic map is provided in Figure 2.

A phylogenetic tree including other disease vector mosquito species is shown in Figure 3. In the phylogenetic tree, the closest match to the whole Mt sequence of *Ae. j. japonicus* was *Ae. koreicus* (GenBank accession number: MT093832) collected from South Korea (Shin and Jung 2020) with 90.81% sequence identity.

Discussion and conclusions

Here, we sequenced and annotated the first complete Mt sequence of wild caught *Ae. j. japonicus* from Hawai'i. The phylogenetic analysis of the whole mitochondria sequence showed that *Ae. j. japonicus* had the closet match to *Ae. koreicus* with 90.81% sequence identify, comparing with any of other co-occurring and morphologically confusing invasive species such as *Ae. albopictus* and *Ae. aegypti* (Wilkerson et al. 2022). This close relationship is consistent with one earlier study that revealed the surprising monophyletic grouping among all subspecies of *Ae. japonicus* and *Ae. koreicus* by using two mitochondrial markers and a nuclear marker (Cameron et al. 2010). Given that these two taxa are invasive sibling species but are difficult to separate morphologically (Tanaka et al. 1979), the results of this study may provide mitochondrial genetic markers for molecular species identification. Data presented here could also have beneficial impacts on future genetic and evolutionary studies on *Ae. japonicus*.

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Ethics statement

The study involves collection of mosquito specimen in public places (beach parks) or private properties. The verbal consent from homeowners was acquired prior to conducting collections in private properties. No permit or ethical approval is required for this study.

Author contributions

YL, CMJ, and OSA conceived experiments; CMJ and YL conducted field collection and species identification; SS, ALR-W, and VTN contributed to sequence generation; SS, ALR-W, VTN, TCC, XW, and MTR contributed to

data analysis; SS, XW, MTR, and YL contributed to drafting the manuscript; all authors contributed to revision of manuscript.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. This publication was developed under Assistance Agreement No. 84020401 awarded by the U.S. Environmental Protection Agency to Akbari. It has not been formally reviewed by EPA. The views expressed in this document are solely those of authors and do not necessarily reflect those of the Agency. EPA does not endorse any products or commercial services mentioned in this publication.

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Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the accession number OP373191. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA882781, SRR21658404, and SAMN30950993, respectively.

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