ANIMAL CELLS AND SYSTEMS
2026, VOL. 30, NO. 1, 146–160
https://doi.org/10.1080/19768354.2026.2620255



# Evolutionary insights from complete mitochondrial genomes of *Aedes albopictus* and *Culex pipiens molestus* (Diptera: Culicidae)

Ashraf Akintayo Akintola[a] and Ui Wook Hwang[a,b,c,d,e]

[a]Department of Biology, Teachers College and Institute for Phylogenomics and Evolution, Kyungpook National University, Daegu, Republic of Korea; [b]Department of Biomedical Convergence Science and Technology, School of Industrial Technology Advances, Kyungpook National University, Daegu, Republic of Korea; [c]Department of Advanced Bioconvergence, Kyungpook National University, Daegu, Republic of Korea; [d]Institute for Korean Herb-Bio Convergence Promotion, Kyungpook National University, Daegu, Republic of Korea; [e]Phylomics Inc., Daegu, Republic of Korea

**ABSTRACT**

Mitochondrial genomes (mitogenomes) are powerful molecular tools for exploring phylogenetic relationships, deciphering adaptation and divergence, as well as disease transmission potential among insects. In this study, we performed a comparative mitogenomic and phylogenetic analysis of two major mosquito vectors – *Aedes albopictus* and *Culex pipiens molestus* – collected from South Korea, together with mitogenome data from 18 additional mosquito species representing three Culicidae genera (*Aedes*, *Anopheles*, and *Culex*) retrieved from GenBank. The assembled mitogenomes, each comprising 13 protein-coding genes, 22 tRNAs, and two rRNAs, revealed conserved gene order and structural features typical of Diptera. Comparative analyses with multiple species representing the three genera uncovered lineage-specific patterns in codon usage, base composition, and control region variability. Phylogenetic relationships inferred using maximum likelihood and Bayesian methods based on concatenated mitochondrial protein-coding genes robustly resolved genus-level relationships and supported the monophyly of the two major Culicidae clades, *Aedes* (tribe Aedini) and *Culex* (tribe Culicini), with *Anopheles sinensis* and *Anopheles gambiae* serving as outgroups. Time-calibrated Bayesian analyses indicated a deep divergence between *Anopheles* and the *Aedes–Culex* clade during the Paleogene (~51.8 Mya), followed by the separation of *Aedes* and *Culex* around ~39.7 Mya. Notably, *Ae. albopictus* diverged from its congeners ~11.7 Mya, while *Cx. p. molestus* exhibited a much more recent divergence (~2.35 Mya), suggesting distinct evolutionary pressures and histories. This study demonstrates the utility of mitogenomic data for reconstructing mosquito evolutionary history and provides insights relevant to vector biology, ecology, and public health.

**ARTICLE HISTORY**
Received 26 April 2025
Revised 4 November 2025
Accepted 14 January 2026

**KEYWORDS**
*Aedes albopictus*; *Culex pipiens molestus*; mitochondrial genome; phylogeny; South Korea;

## Introduction

Mosquitoes (Diptera: Culicidae) are medically important insects that serve as vectors for a wide range of pathogens including arboviruses and parasitic protozoans. Among them, *Aedes albopictus* and *Culex pipiens molestus* have gained global attention due to their wide distribution, adaptability, and roles in transmitting diseases such as dengue, zika, Japanese encephalitis, yellow fever, chikungunya, and West Nile virus (Bhatt et al. 2013; Schaffner et al. 2013). Understanding their evolutionary history and genetic divergence is crucial for disease surveillance and control strategies.

Mitochondrial genomes (mitogenomes), which are distinct cellular organelle that is ubiquitously present in nearly all eukaryotic cells (Osellame et al. 2012; Tao et al. 2014), have been widely employed in phylogenetic and evolutionary studies due to their compact structure, maternal inheritance, and relatively conserved gene content (Harrison 1989; Simon et al. 2006; Li et al. 2022). In most insects, the mitogenome is a double-stranded circular molecule characterized by 22 transfer RNA (tRNA) genes, two ribosomal RNA

**CONTACT** Ui Wook Hwang uwhwang@knu.ac.kr; uwhwang1@gmail.com Department of Biology, Teachers College and Institute for Phylogenomics and Evolution, Kyungpook National University, Daegu 41566, Republic of Korea
Supplemental data for this article can be accessed online at https://doi.org/10.1080/19768354.2026.2620255.

(rRNA) genes, 13 protein-coding genes (PCGs), and a control region (CR) (Boore 1999; Simon et al. 2006; Bernt et al. 2013a). Complete mitogenomic data provide high-resolution markers that improve our ability to reconstruct evolutionary relationships and assess selection pressures across species.

The genus *Aedes* comprises over 950 known species, distributed worldwide, except in Antarctica (Reinert 2000). Only a relatively small subset is of major public-health concern because of their invasiveness and association with arbovirus transmission. Prominent invasive *Aedes* taxa include *A. albopictus, A. aegypti, A. japonicus, A. koreicus,* and *A. atropalpus* (Battaglia et al. 2016). This genus has been extensively studied for its ecological adaptability and vector competence. In contrast, the *Culex pipiens* complex remains taxonomically challenging due to the high morphological similarity and hybridization among its members. This species complex includes *Culex pipiens* Linnaeus 1758 (northern house mosquitoes) and *Cx. quinquefasciatus* Say 1823 (southern house mosquitoes) (Kilpatrick et al. 2005; Fall et al. 2014). *Cx. pipiens,* however, has two biotypes – *Cx. pipiens* f. *pipiens* and *Cx. pipiens* f. *molestus*, both of which are classified based on their autogenic ability, diapause, and blood host preferences (Farajollahi et al. 2011). Other members of the complex are *Cx. p. pallens, Cx. australicus* and *Cx. globocoxitus* (Smith and Fonseca 2004; Harbach 2012; Russell 2012). Members of the *Cx. pipiens* complex are known for their ubiquitous nature in temperate, tropical, and subtropical regions of the world (Knight 1978; Guillemaud et al. 1997; Akintola et al. 2024). Thus, the accurate identification of the mosquito species is a critical step in preventing the spread of mosquito-borne diseases.

Previous mitogenomic studies in mosquitoes have primarily focused on individual species or mitochondrial DNA gene markers such as *COI*, *ND5*, and *CytB*, leaving deeper lineage-level relationships and functional evolution underexplored (Žitko et al. 2011; Beebe et al. 2013; Zhong et al. 2013; Eskildsen et al. 2018). Even though mitochondrial DNA genes are ideal genetic markers to assess ancestry and demographic changes in populations (Avise 1995; Hwang and Kim 1999; Kim et al. 2019, 2024; Choi et al. 2020, 2021; Park et al. 2021; Akintola et al. 2022; Park and Hwang 2022; Choi and Hwang 2023), however, limitations observed from previous population genetic studies with *COI* and *ND5* have suggested that the variation observed may be insufficient for placement of mosquitoes into phylogenetical groups (Torroni et al. 2006; Achilli et al. 2008, 2012).

In this study, we present a comprehensive comparative mitogenomic and evolutionary analysis of *Ae. albopictus* and *C. p. molestus* collected from South Korea. Using 13 protein-coding genes, we assessed genome architecture, codon usage, and nucleotide composition in the context of 18 other mosquito mitogenomes. We reconstructed robust phylogenetic trees using maximum likelihood (ML) and Bayesian inference (BI) approaches and employed a time-calibrated molecular clock to estimate divergence times across key mosquito lineages. Our findings contribute to the understanding of mosquito evolution and diversification, while providing a framework for future studies on vector ecology and control strategies.

## Materials and methods

### *Mosquito collection and DNA extraction*

*Ae. albopictus* and *Cx. p. molestus* samples were collected from domestic, peri-domestic, cow sheds, and small forest areas in different regions in South Korea between February 2021 to September 2022 (Table 1). Light

**Table 1.** Sampling localities and the number of mosquito samples collected in this study.

| Area | Codes | Coordinates (DMS) | No of samples |
|---|---|---|---|
| Haengjeong-ri, Gachang-myeon, Dalseong-gun | HR | 35°53′11.4″ N; 128°36′21.3″ E | *Aedes* – 5<br>*Culex* – 5 |
| Gachang-dong, Sangju-si, Gyeongsangbuk-do | SJ | 36°22′48.5″ N; 128°08′56.9″ E | *Aedes* – 7<br>*Culex* – 22 |
| Yulryang-dong, Cheongwon-gu, Cheongju-si, Chungcheongbuk-do | CM | 36°39′38.7″ N; 127°31′14.7″ E | *Aedes* – 10<br>*Culex* – 4 |
| Gyepo-ri, Cheongtong-myeon, Yeongcheon-si, Gyeongsangbuk-do | CJ | 36°00′03.6″ N; 128°49′04.9″ E | *Aedes* – 10<br>*Culex* – 2 |
| Nam-myeon, Yangju-si, Gyeonggi-do | GR | 37°51′39.6″ N; 126°59′25.3″ E | *Aedes* – 5<br>*Culex* – 3 |
| Daehak-ro, Sangyeok-dong, Buk-gu, Daegu | DG | 35°53′20.8″ N; 128°36′51.6″ E | *Aedes* – 50<br>*Culex* – 150 |

traps and hand-held mosquito nets were used to collect the adult mosquitoes from the sampling locations. Once captured, the mosquitoes were immediately preserved separately in sterile tubes containing 400 µL of DNA/RNA stabilization reagent (Roche, Basel, Switzerland) and/or ethanol. The samples were then transported at room temperature to the laboratory for species identification. Species identification was conducted using standard morphological keys and diagnostic characters following the taxonomic criteria outlined by Rueda (2004) and Harbach (2012), with an ordinary microscope (Olympus, Tokyo, Japan). Each mosquito was removed from the DNA/RNA stabilization reagent and placed onto a glass slide for morphological identification. After species and gender identification, the mosquitoes were stored at −80° C until genomic DNA extraction. Total genomic DNA was extracted from thoracic tissues of morphologically identified adult mosquitoes using a DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. In contrast, the legs of each specimen were used exclusively for molecular identification targeting the *COI*, *16S rRNA*, and *ace-2* gene regions to minimize the risk of contamination from non-host DNA.

Due to the difficulty in resolving the molecular identity of members of the *Cx. pipiens* complex with *16S rRNA*, the acetylcholinesterase 2 (*ace 2*) region using universal forward primers developed by Smith and Fonseca (2004) and species-specific reverse primers by Ryu and Choi (2022) were utilized to distinguish between them (Table S1).

The complete mitochondrial genome of *Cx. p. molestus* was thereafter amplified using 18 primer pairs designed by Zhang et al. (2013). For the complete mitochondrial genome sequencing of *Aedes albopictus*, 18 primer pairs designed by Ze-Ze et al. (2020) were used to amplify overlapping fragments covering the entire mitochondrial genome. All amplified PCR products were checked on 1.0% agarose gel and purified using a QIAquick PCR Purification Kit (Qiagen Co., Hilden, Germany).

## Mitochondrial genome sequencing and annotation

The amplicons were sequenced directly using an ABI Prism 3730 DNA sequencer (PerkinElmer, Waltham, MA) with a BigDye Termination Sequencing Kit. All alignments were performed in the Clustal X2 program (Larkin et al. 2007) and BioEdit 7.0.9 program (Hall 1999). The annotation of the mitogenome was conducted using the MITOS Web server to identify the PCGs, rRNA genes, and tRNA genes, including their locations and the secondary structures of tRNAs (Bernt et al. 2013b). The annotation results were confirmed by using NCBI Basic Local Alignment Search Tool (BLAST) comparison with the respective homologous sequences of other *Aedes,* and *Culex* species downloaded from GenBank, tRNAscan-SE and ARWEN v1.2 online program (Laslett and Canbäck 2008; Chan and Lowe 2019). Overlapping and intergenic spacer regions were calculated manually. Mega X was used to analyze the characteristics of the mitogenome, including sequence length, base content, codon usage, and amino acid composition. The AT skewness and GC skewness were, respectively, determined by the following formula: AT skew = (A − T)/(A + T) and GC skew = (G − C)/(G + C). The tandem repeat (TR) sequences in the control region were examined using the Tandem Repeats Finder Program (https://tandem.bu.edu/trf/trf.html) (Benson 1999).

## Phylogenetic analyses

For comparative and phylogenetic analyses, complete mitochondrial genomes from 18 additional mosquito species were retrieved from GenBank, representing the genera *Aedes*, *Culex*, and *Anopheles* within the family Culicidae. These included *Ae. aegypti*, *Ae. japonicus*, *Ae. koreicus*, *Ae. busckii*, *Ae. flavopictus*, *An. gambiae*, *An. sinensis*, *Cx. quinquefasciatus*, *Cx. p. pipiens*, *Cx. p. pallens*, *Cx. tritaeniorhynchus*, as well as *Cx. sitiens*. All sequences were downloaded from GenBank on 15 January 2024 and verified for completeness and annotation consistency prior to analysis.

The phylogenetic tree was constructed using concatenated sequences of 13 mitochondrial protein-coding genes from representative species of the genera – *Aedes* and *Culex,* within the family *Culicidae*. *An. sinensis* and *An. gambiae* were used as the outgroup. Clustal X2 (Larkin et al. 2007) was used in aligning the PCG amino acid sequences. The best-fit nucleotide substitution model was determined using jModelTest v2.1.10 (Darriba et al. 2012) based on the Akaike Information Criterion (AIC) and applied consistently in both

ML and BI analyses. Using the maximum likelihood (ML) method, the phylogenetic tree was built with 1000 bootstrap replicates and substitution model GTR + F + I + G4, through IQ-TREE webserver (Trifinopoulos et al. 2016). Bayesian inference of phylogenetic relationships was conducted using BEAST v2.7.x (Bouckaert et al. 2019). The analysis was performed under an uncorrelated lognormal relaxed clock model to account for rate variation among lineages. A Yule speciation process was used as the tree prior. The MCMC (Markov Chain Monte Carlo) chain was run for 100 million generations, with parameters logged every 10,000 generations. To ensure sufficient sampling, two independent BEAST runs were performed and later combined. All BEAST XML input files were generated using BEAUti, the graphical interface provided with BEAST. Tracer v1.7.2 (Rambaut et al. 2018) was used to assess convergence and ensure all parameters had effective sample size (ESS) values >200. The resulting log and tree files were combined using LogCombiner (with a burn-in of 10%) and summarized using TreeAnnotator to generate the maximum clade credibility (MCC) tree. The final tree was visualized and edited in FigTree v1.4.4. To estimate the selective pressure acting on mitochondrial genes, we computed the dN/dS ratio for each PCG using the CODEML module of PAML v4.9 (Yang 2007). Bayesian inference was used to determine whether specific codons were under positive selection. A dN/dS ratio <1 indicated purifying selection, a ratio ≈1 suggested neutral evolution, and a ratio >1 implied positive selection.

### *Divergence time estimation*

Divergence time estimation for the 20 mosquito species was conducted using mitochondrial genome sequences in BEAST 2.5 (Bouckaert *et al*., 2019). The *Culicidae* root age was constrained at ∼100 Mya (normal distribution, SD = 10), thus we calibrated the Culicidae clade at 75 million years ago (Ma) based on the oldest mosquito fossils from the mid-cretaceous and previous studies on divergence times in dipteran and mosquito evolution studies (Reidenbach et al. 2009; Wiegmann et al. 2011). The analysis employed a relaxed log-normal molecular clock model and a Birth Death speciation prior, which is suitable for modeling interspecies diversification. The GTR + F + G4 substitution model was used for tree construction after being determined ModelFinder in IQ-TREE v2.2.0. The analysis was executed for 10,000,000 MCMC iterations and sampled every 1000 iterations, after discarding the initial 10% of iterations as burn-in. The final divergence time tree was visualized using FigTree v1.4.4.

## Results

### *Mitochondrial genome characterization of* Aedes albopictus *and* Culex pipiens molestus

The complete mitochondrial genomes of *Ae. albopictus* (16,816 bp; PQ584793) and *Cx. p. molestus* (15,636 bp; PQ584792) contain 37 genes – 13 protein-coding genes (PCGs), 22 *tRNAs*, and 2 *rRNAs* – organized in the ancestral arthropod order. Most genes are encoded on the heavy strand, with exceptions (e.g. *ND1*, *ND4*, *ND4L*, *ND5*, *rrnL*, *rrnS*, and some *tRNAs*) on the light strand. *A. albopictus* possesses a 1,920 bp control region (CR), while the size of the CR in *Cx. pipiens molestus* is 780 bp, both between *rrnS* and *trnI*. Further details on gene features are provided in Table 2 and Figure 1.

Both mitogenomes show a strong A + T bias (*Ae. albopictus*: 80.0%; *Cx. p. molestus*: 77.7%) (Table S2 and S3) and similar AT- and GC-skew patterns typical of mosquitoes. Start codons across PCGs include ATG, ATT, or ATA, with incomplete stop codons (T or TA) found in some genes, a common feature in insects. Codon usage favors A/T-rich codons like TTT, ATT, and AAT (Table S4 and S5). The 22 *tRNAs* range from 64 to 72 bp, most forming typical cloverleaf structures except *trnS1*, which lacks a DHU arm. The *rRNA* genes (*rrnL* and *rrnS*), located on the light strand and separated by *trnV*, show conserved structure. The CR in both species is highly AT-rich (92.2% in *A. albopictus* and 88.6% in *Cx. pipiens molestus*: 77.7%) (Table S2 and S3) and includes several repeats and non-coding elements, consistent with other Culicidae mitogenomes (Krzywinski et al. 2011; do Nascimento et al. 2021). The codon usage analysis revealed that both mitogenomes preferentially use codons ending in A or T, reflecting the AT-rich nature of insect mitochondrial genomes (Boore 1999; Cameron 2014). The most frequently used codons were UUA (Leu), AUU (Ile), and UUU (Phe). Relative synonymous codon usage (RSCU) patterns were similar between the two species, although slight differences in certain leucine and serine codons were observed (Figure S1).

**Table 2.** Organization of the *Aedes albopictus* and *Culex pipiens molestus* mitochondrial genome

| S/N | *A.albopictus* Gene | Position Start | Position Start | Size | Strand | Anticodon | Start codon | Stop codon | No of intergenic nucleotides | *Cx.p.molestus* Gene | Position Start | Position Stop | Size | Strand | Anticodon | Start codon | Stop codon | No of intergenic nucleotides |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | tRNAIle | 1 | 69 | 69 | + | GAT | | | | tRNAIle | 1 | 69 | 69 | + | GAT | | | |
| 2 | tRNAGln | 71 | 139 | 69 | - | TTG | | | 1 | tRNAGln | 70 | 138 | 69 | - | TTG | | | 0 |
| 3 | tRNAMet | 139 | 207 | 69 | + | CAT | | | -1 | tRNAMet | 142 | 210 | 69 | + | CAT | | | 3 |
| 4 | ND2 | 208 | 1233 | 1026 | + | | ATT | TAA | 0 | ND2 | 211 | 1233 | 1023 | + | | ATC | TAA | 0 |
| 5 | tRNATrp | 1234 | 1303 | 70 | + | TCA | | | 0 | tRNATrp | 1235 | 1303 | 69 | + | TCA | | | 1 |
| 6 | tRNACys | 1303 | 1369 | 67 | - | GCA | | | -1 | tRNACys | 1304 | 1369 | 66 | - | GCA | | | 0 |
| 7 | tRNATyr | 1370 | 1435 | 66 | - | GTA | | | 0 | tRNATyr | 1382 | 1447 | 66 | - | GTA | | | 12 |
| 8 | COI | 1434 | 2970 | 1537 | + | | TCG | T | -2 | COI | 1446 | 2982 | 1537 | + | | TCG | T | -2 |
| 9 | tRNALeu | 2971 | 3037 | 67 | + | TAA | | | 0 | tRNALeu | 2983 | 3049 | 67 | + | TAA | | | 0 |
| 10 | COII | 3039 | 3723 | 685 | + | | ATG | T | 1 | COII | 3055 | 3739 | 685 | + | | ATG | T | 5 |
| 11 | tRNALys | 3724 | 3794 | 71 | + | CTT | | | 0 | tRNALys | 3740 | 3810 | 71 | + | CTT | | | 0 |
| 12 | tRNAAsp | 3807 | 3876 | 70 | + | GTC | | | 12 | tRNAAsp | 3821 | 3888 | 68 | + | GTC | | | 10 |
| 13 | ATP8 | 3877 | 4038 | 162 | + | | ATT | TAA | 0 | ATP8 | 3898 | 4050 | 153 | + | | ATA | TAA | 9 |
| 14 | ATP6 | 4032 | 4712 | 681 | + | | ATG | TAA | -7 | ATP6 | 4044 | 4724 | 681 | + | | ATG | TAA | -7 |
| 15 | COIII | 4716 | 5504 | 789 | + | | ATG | TAA | 3 | COIII | 4724 | 5511 | 788 | + | | ATG | TAA | -1 |
| 16 | tRNAGly | 5504 | 5570 | 67 | + | TCC | | | -1 | tRNAGly | 5512 | 5578 | 67 | + | TCC | | | 0 |
| 17 | ND3 | 5571 | 5924 | 354 | + | | ATA | TAA | 0 | ND3 | 5576 | 5930 | 355 | + | | AGA | T | -3 |
| 18 | tRNAArg | 5923 | 5986 | 64 | + | TCG | | | -2 | tRNAArg | 5931 | 5994 | 64 | + | TCG | | | 0 |
| 19 | tRNAAla | 5987 | 6054 | 68 | + | TGC | | | 0 | tRNAAla | 5995 | 6060 | 66 | + | TGC | | | 0 |
| 20 | tRNAAsn | 6056 | 6122 | 67 | + | GTT | | | 1 | tRNAAsn | 6061 | 6127 | 67 | + | GTT | | | 0 |
| 21 | tRNASer | 6121 | 6187 | 67 | - | CTA | | | -2 | tRNASer | 6126 | 6190 | 65 | - | CTA | | | -2 |
| 22 | tRNAGlu | 6205 | 6270 | 66 | + | TTC | | | 17 | tRNAGlu | 6198 | 6263 | 66 | + | TTC | | | 7 |
| 23 | tRNAPhe | 6269 | 6335 | 67 | - | GAA | | | -2 | tRNAPhe | 6262 | 6328 | 67 | - | GAA | | | -2 |
| 24 | ND5 | 6343 | 8085 | 1743 | - | | TTA | T | 7 | ND5 | 6329 | 8074 | 1746 | - | | TAT | TAA | 0 |
| 25 | tRNAHis | 8086 | 8154 | 69 | - | GTG | | | 0 | tRNAHis | 8072 | 8137 | 66 | - | GTG | | | -3 |
| 26 | ND4 | 8157 | 9500 | 1344 | - | | TTA | T | 2 | ND4 | 8138 | 9480 | 1343 | - | | TTT | TA | 0 |
| 27 | ND4L | 9494 | 9790 | 297 | - | | ATA | T | -7 | ND4L | 9474 | 9770 | 297 | - | | GTT | TAA | -7 |
| 28 | tRNAThr | 9793 | 9857 | 65 | + | TGT | | | 2 | tRNAThr | 9776 | 9840 | 65 | + | TGT | | | 5 |
| 29 | tRNAPro | 9858 | 9924 | 67 | - | TGG | | | 0 | tRNAPro | 9841 | 9906 | 66 | - | TGG | | | 0 |
| 30 | ND6 | 9927 | 10448 | 522 | + | | ATT | TAA | 2 | ND6 | 9912 | 10427 | 516 | + | | ATT | TAA | 5 |
| 31 | CYTB | 10448 | 11584 | 1137 | + | | ATG | TAA | -1 | CYTB | 10427 | 11561 | 1135 | + | | ATG | T | -1 |
| 32 | tRNASer | 11584 | 11649 | 66 | + | TGA | | | -1 | tRNASer | 11562 | 11627 | 66 | + | TGA | | | 0 |
| 33 | ND1 | 11672 | 12613 | 942 | - | | TAT | TAT | 22 | ND1 | 11646 | 12602 | 957 | - | | TAT | TAA | 18 |
| 34 | tRNALeu | 12623 | 12690 | 68 | - | TAG | | | 9 | tRNALeu | 12599 | 12663 | 65 | - | TAG | | | -4 |
| 35 | lrRNA | 12692 | 14026 | 1335 | - | | | | 1 | lrRNA | 12667 | 13999 | 1333 | - | | | | 3 |
| 36 | tRNAVal | 14028 | 14099 | 72 | - | TAC | | | 1 | tRNAVal | 14000 | 14071 | 72 | - | TAC | | | 0 |
| 37 | srRNA | 14100 | 14896 | 797 | - | | | | 0 | srRNA | 14072 | 14856 | 785 | - | | | | 0 |
| 38 | CR | 14897 | 16816 | 1920 | + | | | | 0 | CR | 14857 | 15636 | 780 | + | | | | 0 |

## *Selection pressure analysis*

To understand the evolutionary dynamics of mitochondrial genes, we estimated the ratio of nonsynonymous to synonymous substitutions (dN/dS) for each PCG across 20 mosquito species (Table S6). The dN/dS values indicate the degree of selective pressure acting on mitochondrial genes (Yang and Nielsen 2000). Among the 13 PCGs, *ATP8* exhibited the highest dN/dS ratio (0.1700), suggesting it experiences relatively relaxed

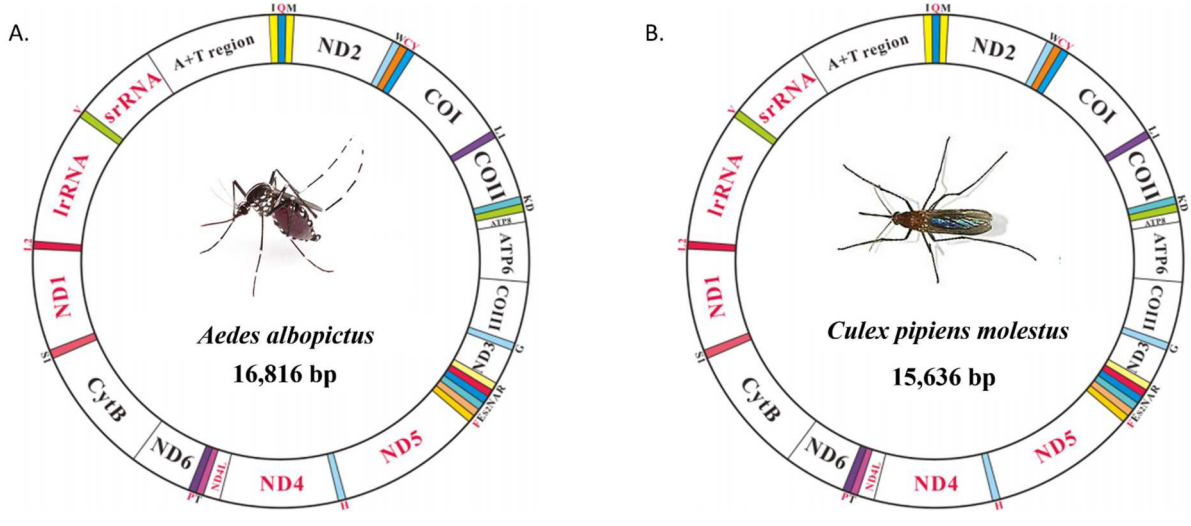


**Figure 1.** Structure of the (A) *Aedes albopictus* and (B) *Culex pipiens molestus* mitochondrial genome. The color-filled blocks indicate tRNAs, and the unfilled white blocks denote protein-coding genes (PCGs), rRNAs and control region (CR). The tRNAs, PCGs, rRNAs and CR located on the majority strand are marked in black, and those on the minority strand are marked in red.

purifying selection or potential functional divergence. In contrast, *ND4L* and *ND6* showed a dN/dS ratio of zero, indicating extreme conservation and strong purifying selection. The COX and ND gene families generally had very low dN/dS values (<0.006), reflecting high functional constraints on oxidative phosphorylation and energy metabolism. These findings are consistent with previous studies on mitochondrial evolution, which report that mitochondrial PCGs are subject to strong purifying selection to maintain efficient cellular respiration and ATP production (Bernt *et al*., 2013a,b).

## *Phylogenetic relationships within Culicidae*

A phylogenetic tree was constructed using maximum likelihood (ML) and Bayesian inference (BI) based on the concatenated sequences of the 13 PCGs from *Ae. albopictus*, *Cx. p. molestus*, and 18 other Culicidae species obtained from GenBank (Figure 2). Both ML and BI trees yielded consistent topologies, strongly supporting the monophyly of the *Aedes*, *Culex*, and *Anopheles* genera.

Phylogenetic analyses also well supported *Aedes* and *Culex* as sister taxa, thus placing both genera in the Aedini and Culicini tribes, in accordance with the taxonomic classification proposed by Krzywinski et al. (2011). It is also reported by previous studies that Aedini was a sister group of Culicini and the divergence observed in the two group occurred 130 mya ago (Reidenbach et al. 2009; do Nascimento et al. 2021). *Ae. albopictus* clustered closely with *Ae. aegypti*, while *Cx. p. molestus* grouped with *Cx. quinquefasciatus* and *Cx. p. pipiens*, confirming their close evolutionary relationships within their respective clades. The divergence

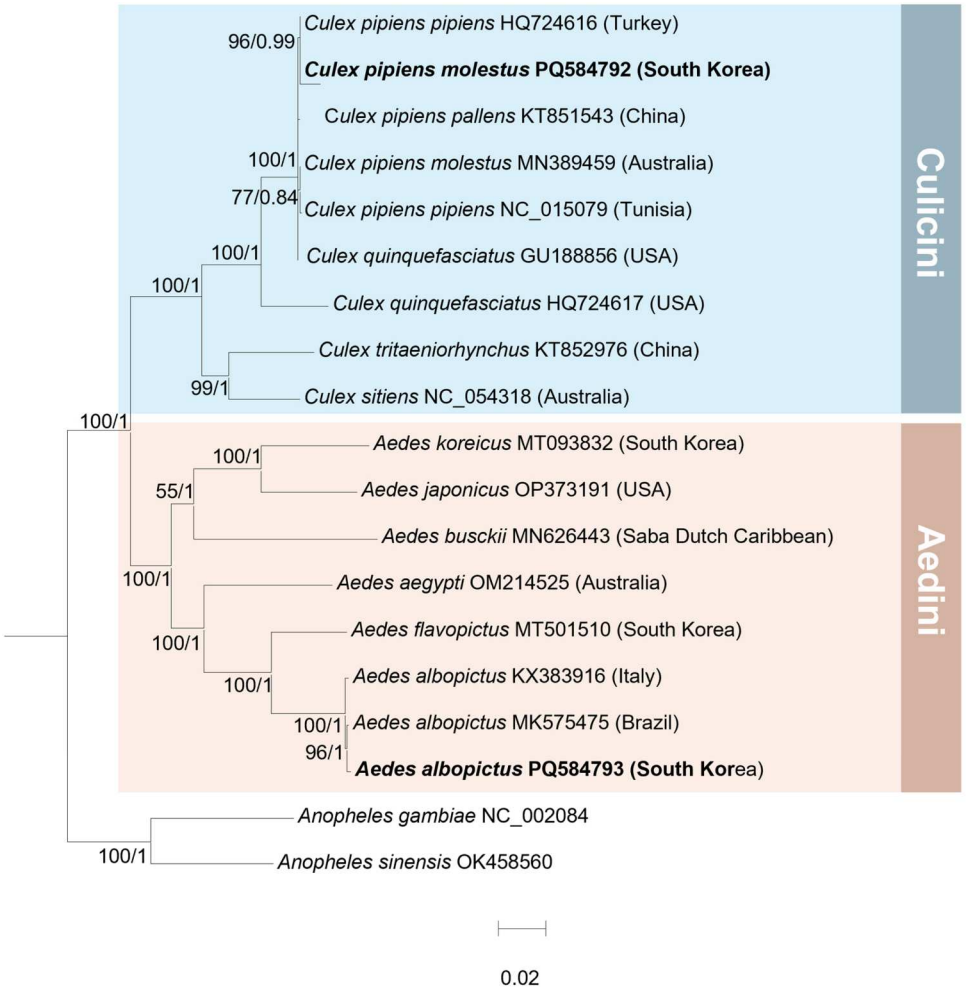


**Figure 2.** A maximum likelihood (ML) tree reconstructed based on nucleotide sequences of the 13 mitochondrial PCGs. Each node showed the bootstrap value for ML analysis/Bayesian posterior probability. A newly sequenced *Aedes albopictus* and *Culex pipiens molestus* from South Korea were highlighted in dark bold colors.

between the *Aedes* and *Culex* lineages was evident, reflecting their ecological and evolutionary separation. Bootstrap and posterior probability values for key nodes were consistently above 90% and 0.95, respectively (Figure 2 and S2). According to recent classification reports, the genus *Aedes* has been divided into several subgenera, such as *Aedes*, *Diceromyia*, *Finlaya*, and *Stegomyia*. Most of these subgenera have been treated by some authorities as full genera (Reinert et al. 2004, 2009).

Intraspecific percentage identity variation analysis over the entire mitogenome (Table S7) for *Aedes albopictus* showed a surprisingly low intraspecific variation (0.14%) between the South Korean and Italian samples, suggesting recent common ancestry or strong selective constraints maintaining mitogenome conservation across these regions. Conversely, the relatively higher genetic divergence between the South Korean and Brazilian samples (3.29%), and even slightly more between Brazil and Italy (3.32%), indicates a more substantial degree of mitogenomic differentiation. These values suggest that the Brazilian sample may have derived from a separate introduction event or has since undergone genetic drift, local adaptation, or population bottlenecks, resulting in increased divergence from both European and Asian lineages. This pattern supports findings from previous global phylogeographic studies indicating that *A. albopictus* introductions into the Americas may have originated from different source populations compared to those in Europe (Kotsakiozi et al. 2017).

As shown in Figure 2, the major clades on the ML and BI tree supported the monophylies of the *Culex* genera. *Cx. pipiens molestus* from South Korea, based on concatenated mitochondrial protein-coding genes (PCGs) revealed a close clustering of *Cx. p. molestus* from South Korea and *Cx. p. pipiens* from Turkey (HQ724616). Analysis of the intraspecific variation across the whole mitogenome (Table S7) also revealed a low sequence divergence (0.03%) between these two samples, suggesting a recent common maternal lineage or the possibility of gene flow between these populations. This supports previous findings that mitochondrial markers can sometimes fail to distinguish between ecoforms due to introgression and hybridization (Shaikevich et al. 2016).

Contrasting this close relationship is the more substantial divergence (0.28%) between *Cx. pipiens molestus* specimens from South Korea and Australia (Table S7), both bearing the same taxonomic label. This raises questions about the monophyly of the *molestus* form, which may not represent a single evolutionary lineage but rather a convergent ecological adaptation (e.g. autogeny, stenogamy, underground breeding) across different regions (Fonseca et al. 2004). Similarly, the divergence between the Turkish and Tunisian *Cx. p. pipiens* specimens (0.17%) reflect regional genetic structuring and adaptation to local environments.

The greatest divergence was observed between *Cx. p. pallens* (China) and *Cx. quinquefasciatus* (USA) at 1.07%, aligning with either the accepted view that *Cx. quinquefasciatus* is a genetically distinct species despite its ability to hybridize with members of the *pipiens* complex in areas of sympatry or a misidentification of the species as a *Cx. quinquefasciatus* sample. On the first reason, this level of divergence further underscores the distinct evolutionary trajectory of *quinquefasciatus* and supports its taxonomic separation from the *pipiens* clade. Alternatively, it might be due to the insufficient tools of the researchers to distinguish between members of the *Culex* species complex. We also faced a similar issue of misidentification until we used a species-specific primer reported earlier to show the distinction between members of the species complex. Tables S8 and S9 also show the intraspecific nucleotide diversity across different positions in the mitogenome of *Ae. albopictus* complex and *Culex pipiens* species complex, respectively.

## Control region analysis

The mitochondrial control region (CR), the major non-coding segment responsible for regulating replication and transcription of mitochondrial DNA, displayed marked differences in length, structure, and sequence composition between *Ae. albopictus* and *Cx. p. molestus*. In *Ae. albopictus*, the control region was located between the 12S rRNA and tRNA-Ile genes and spanned 1,920 bp. This region exhibited a relatively high A + T content (92.2%), consistent with previously reported patterns in mosquito mitogenomes. Notably, we identified multiple tandem repeat units in this region, consisting of a highly conserved 190 bp motif repeated in tandem six times (Figure 3). These repeat units exhibited no sequence divergence among the South Korean and Italian samples, thus suggesting recent duplication events. The presence of extensive tandem repeats likely contributes to the variability in CR length and may play functional roles in mtDNA replication and stability.

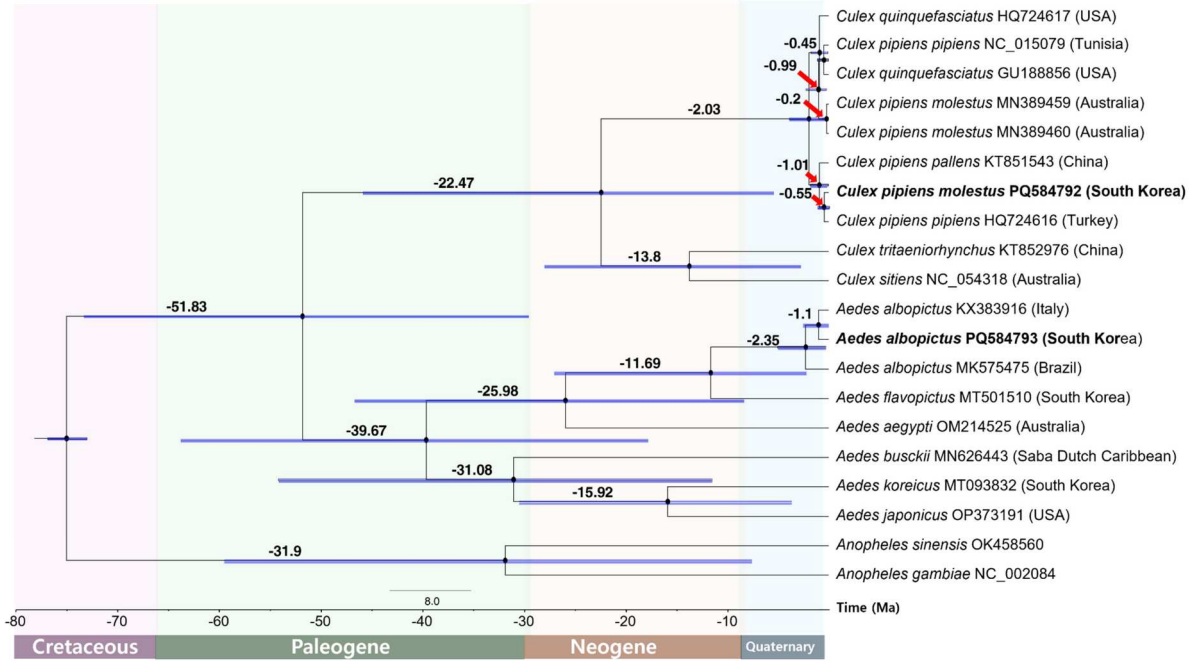


**Figure 3.** Divergence time estimation based on 13 PCGs from 20 mosquito species. The tree was constructed using BEAST2 with fossil-based calibration points, and divergence times are shown in millions of years ago (Ma) at each node. The 95% highest posterior density (HPD) intervals are represented by horizontal blue bars, indicating uncertainty in divergence time estimates. The geological time scale (Cretaceous, Paleogene, Neogene, and Quaternary) is shown in color blocks along the x-axis. The red arrows point only to the divergence time positions in the tree branch.

Additionally, several poly-T stretches and AT-rich motifs, commonly associated with the initiation of replication in Dipteran insects, were observed. We also detected stem-loop structures using the Mfold web server, with predicted ΔG values indicating thermodynamically stable secondary structures. These may serve as origins of replication or transcription regulation elements.

In contrast, the control region in *Cx. p. molestus* was 780 bp in length, which is almost half the size of that in *Ae. albopictus* and located between the 12S *rRNA* and *tRNA-Ile* genes as well. The A + T content was slightly lower at 88.6%. Unlike *Aedes albopictus*, no long tandem repeats were observed in the *Culex* CR (Figure S3). Instead, the region was composed of several short microsatellite-like sequences. The structural simplicity and reduced length of the *Culex* CR may reflect lineage-specific evolutionary dynamics, possibly influenced by different selective pressures or mutational mechanisms. While both species share similar gene arrangements flanking the CR, their divergent sequence architectures underscore the role of the control region in lineage-specific mitochondrial genome evolution.

## *Divergence time estimate*

To investigate the evolutionary timeline of major mosquito lineages, we performed Bayesian divergence time estimation using 13 concatenated mitochondrial protein-coding genes (PCGs) from 20 species representing the genera *Aedes*, *Culex*, and *Anopheles*. The resulting maximum clade credibility (MCC) tree is shown in Figure 4, with node bars indicating the 95% Highest Posterior Density (HPD) intervals for divergence times. The root of the Culicidae tree was estimated to have occurred approximately 51.83 million years ago (Mya) [95% HPD: ~73.00–29.00 Mya], during the Paleogene period, following an earlier diversification event likely initiated in the Cretaceous. The basal split separated the *Anopheles* lineage from the common ancestor of *Culex* and *Aedes*. This result supports previous phylogenomic findings suggesting the early divergence of *Anopheles* (Reidenbach et al. 2009).

The divergence between *Aedes* and *Culex* lineages was inferred to have occurred at approximately 39.67 Mya (95% HPD range spanning the Paleogene), indicating a long evolutionary history for these medically significant genera. Within the *Culex* clade, *Cx. p. molestus* was shown to diverge from its closest relatives

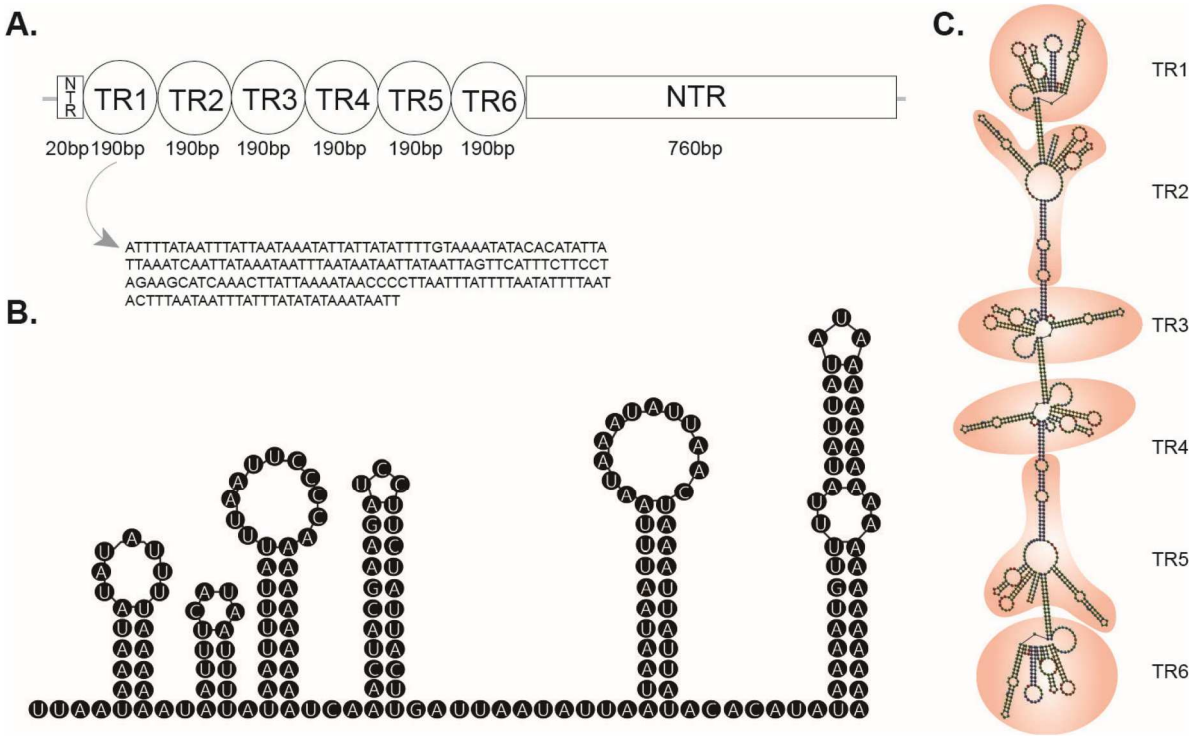


**Figure 4.** Structure and arrangement of *Aedes albopictus* mitochondrial control region. (A) Schematic representation of the control region showing the arrangement of tandem repeats (TR1–TR6) and the adjacent non-tandem repeat (NTR) region. Each tandem repeat unit is approximately 190 bp in length, followed by a 760 bp NTR segment. The consensus nucleotide sequence of one tandem repeat unit is displayed beneath the schematic. (B) Predicted secondary structure of a single tandem repeat unit, highlighting conserved stem-loop motifs. (C) Predicted secondary structures for all six tandem repeats (TR1–TR6), demonstrating repeated and potentially conserved structural motifs across repeat units.

around 2.35 Mya, during the Quaternary period, suggesting relatively recent speciation possibly linked to anthropogenic changes in urban environments. The divergence of *Ae. albopictus* from its closest sampled congeners was estimated at 11.69 Mya, within the Neogene period, indicating a much older divergence time than *Culex pipiens molestus*. This suggests different evolutionary dynamics and possibly adaptive pressures in shaping their current distribution and ecological niches. Notably, most diversification events within *Culex* occurred more recently, within the last 2–3 million years, whereas *Aedes* species exhibit deeper divergence times, consistent with broader geographic and ecological expansion. The majority of *Anopheles* nodes diverged earlier, between 30 and 50 Mya, further supporting the basal positioning of the genus in mosquito phylogeny.

## Discussion

The mitochondrial genome analysis and phylogenetic reconstruction presented in this study provide critical insights into the evolutionary history and divergence of *Ae. albopictus* and *Cx. p. molestus* from South Korea as well as other related mosquito taxa. Our results confirmed the conserved organization of mosquito mitogenomes but also highlight the lineage-specific variations, particularly in the control region and codon usage patterns, which are consistent with previously observed adaptive trends in Culicidae evolution. This organization supports the evolutionary conservation of mitochondrial architecture across Diptera and suggests that major rearrangements are rare within Culicidae, likely due to functional constraints associated with replication and transcriptional regulation of the mitochondrial genome. Comparative analyses of gene order across mosquito lineages further confirm that both *Aedes* and *Culex* retain the ancestral Culicidae arrangement, consistent with previously reported mitogenomes from *Anopheles*, *Armigeres*, *Ochlerotatus*, and *Tripteroides* species (Krzywinski et al. 2011; Zhang et al. 2013; do Nascimento et al. 2021). The high degree of structural conservation across these taxa implies that mitochondrial genome rearrangements

have had limited phylogenetic utility at the genus level within mosquitoes, although subtle variations in intergenic spacer lengths and control region structures may reflect lineage-specific evolutionary dynamics. The preservation of the Dipteran gene order pattern in both *A. albopictus* and *Cx. pipiens molestus* reinforces their placement within the subfamilies Culicinae and the tribes Aedini and Culicini, respectively, supporting the broader monophyly of Culicidae inferred from mitochondrial phylogenomic data.

The whole mitochondrial genome-based phylogenetic tree reconstructed using maximum likelihood and Bayesian inference methods reveals well-supported clades corresponding to major mosquito lineages. *A. albopictus* and *Cx. pipiens molestus* are placed in distinct monophyletic groups within the Aedini and Culicini tribes, respectively, confirming their taxonomic validity. Notably, the divergence between these two species is marked by distinct mitochondrial features, including differences in genome size, A + T content, and the presence of a control region. Codon usage analysis reveals a strong A + T bias across both species, with a preference for A/T-ending codons. This may indicate selection for translational efficiency or mutational bias shaped by mitochondrial replication and transcription mechanisms. The presence of incomplete stop codons in several PCGs may represent translational adaptations conserved across insect mitochondrial genomes.

The phylogenetic placement of *Cx. p. molestus* suggests a relatively recent divergence from *Cx. p. pipiens*, consistent with ecological niche specialization in urban environments. This supports previous hypotheses that *Cx. p. molestus* may have evolved through peripatric speciation and adaptation to subterranean habitats. Meanwhile, *A. albopictus* maintains a basal yet distinct position within Aedes, reinforcing its ancestral spread and subsequent global radiation.

Phylogenetically, the mitogenomes of *Aedes* species obtained in this study align with previous studies on the Culicidae family (Battaglia et al. 2016). Our phylogenetic trees based on concatenated protein-coding genes (PCGs) positioned the South Korean *Ae. albopictus* closely with Brazilian samples, with the Italian sample more distantly related. This clustering, while counterintuitive based on geographic distances, reflects the mosquito's complex invasion history and human-facilitated spread via trade routes like used tire shipments (Medlock et al. 2012; Bonizzoni et al. 2013). Surprisingly, genetic distance analysis contradicted this tree topology: Brazil was more divergent from both South Korea and Italy, despite clustering with the former. This discrepancy suggests that mitochondrial evolution in *Ae. albopictus* is shaped by both lineage sorting and selective pressures acting across varying timescales and illustrates how mitogenomes may reflect historical demography rather than current nuclear gene flow (Battaglia et al. 2016).

Importantly, the low divergence (0.14%) between South Korean and Italian *Ae. albopictus* supports a recent common ancestry or strong purifying selection, consistent with the known intraspecific variation threshold for mosquitoes. This mitogenomic conservation likely contributes to the species' adaptability and resilience, aiding its colonization of diverse climates and continued role as a global vector of arboviruses like dengue, chikungunya, and Zika.

The phylogenetic analysis of *Cx. p. molestus* adds further complexity. The *Culex pipiens* complex comprises several morphologically similar taxa that are genetically and ecologically distinct, often capable of hybridization. Using maximum likelihood (ML) and Bayesian inference (BI) based on concatenated PCGs, our analysis positioned the South Korean *Cx. p. molestus* closely with *Cx. p. pipiens* from Turkey, with a negligible mitogenomic divergence of 0.03%, suggesting recent maternal ancestry or introgression. Conversely, a divergence of 0.28% was noted between the Korean *molestus* and an Australian *molestus*, indicating possible cryptic diversity or convergent ecological traits, such as autogeny and underground breeding, that have evolved independently (Fonseca et al. 2004).

Furthermore, divergent patterns among other *Cx. pipiens* members, such as *Cx. p. pipiens* (Turkey vs. Tunisia, 0.17%) reveals regional genetic structuring. The highest divergence (1.07%) occurred between *Cx. p. pallens* (China) and *Cx. quinquefasciatus* (USA), supporting the taxonomic distinction of *quinquefasciatus* within the complex, despite its hybridization capacity in zones of sympatry. However, such divergence might also arise from misidentification – a challenge we also faced during our study until species-specific primers helped delineate complex members. These findings underscore the limitations of mitochondrial markers alone in species delimitation within the *Culex pipiens* complex, as introgression and hybridization may obscure lineage boundaries (Shaikevich et al. 2016). Hence, while mitochondrial genomes are powerful for understanding maternal lineage, they must be interpreted alongside nuclear markers such as microsatellites or SNPs to resolve phylogenetic uncertainties.

Consequently, the divergence time tree indicates that the most recent common ancestor (MRCA) of the studied mosquito species dates back to approximately 51.83 million years ago (Mya), during the early Eocene epoch of the Paleogene period. This timing coincides with a period of global warming and habitat transformation following the Cretaceous–Paleogene (K–Pg) extinction event (~66 Mya), which likely contributed to the early diversification of dipteran insects, including mosquitoes (Wiegmann et al. 2011). Our tree topology suggests that the *Aedes*, *Culex*, and *Anopheles* lineages had already emerged by the mid-Paleogene, with subsequent radiation occurring through the Neogene and Quaternary. The divergence between *Aedes* and *Culex* genera is estimated to have occurred around 39.67–31.08 Mya, supporting previous molecular estimates that suggest major mosquito clades diversified in concert with the expansion of angiosperms and the rise of mammals (Reidenbach et al. 2009).

*Aedes albopictus*, the Asian tiger mosquito, diverged from its nearest relatives approximately 11.69 Mya, during the Miocene epoch of the Neogene period. The Miocene was characterized by climatic cooling and forest fragmentation, which likely exerted selective pressures favoring ecological versatility (Zachos et al. 2001). This flexibility is reflected in the modern-day *Ae. albopictus*, which is capable of surviving in both tropical and temperate environments and breeding in a variety of artificial containers. Its ability to adapt to anthropogenic landscapes has contributed to its recent global expansion and increased epidemiological importance (Benedict et al. 2007). Likewise, the divergence time between the Euro-Asian (Italy and South Korea) and South American (Brazil) samples of *Ae. albopictus* indicates a very recent dispersal and differentiation which might indicate a human-mediated introduction of *Ae. albopictus* to South America from Asia, which is likely during global trade expansion.

In contrast, *Cx. p. molestus* diverged more recently – approximately 1.1–2.35 Mya – during the Quaternary period, which encompasses multiple glacial and interglacial cycles. The shallow divergence suggests that *Cx. p. molestus* may be a product of recent adaptive radiation, potentially driven by human activity and urbanization. This taxon is known for its subterranean breeding behavior, autogeny, and lack of diapause, traits believed to be adaptations to life in underground human-made structures such as basements and sewers (Vinogradova 2000; Fonseca et al. 2004). These traits distinguish *Cx. p. molestus* from their conspecific *Cx. p. pipiens*, highlighting the potential for ecological divergence within vector species that occupy human-associated niches. Such divergence, though recent, can have profound implications for disease transmission, especially in densely populated urban centers. The contrasting divergence times and ecological trajectories of *Ae. albopictus* and *Cx. p. molestus* reflects the complex interplay between evolutionary history, environmental change, and human influence. While *Ae. albopictus* exhibits traits of long-term adaptive radiation and global invasion potential, *Cx. p. molestus* represents a model for studying recent adaptation to urban environments.

Understanding the evolutionary history of these vectors is crucial for predicting their future distribution and vectorial capacity, particularly in the face of climate change and expanding urbanization. Our findings underscore the need for targeted vector control strategies that consider not just species-level identity, but also evolutionary and ecological context.

A notable feature of the *Ae. albopictus* CR is the presence of six tandem repeats (Figure 3) structures that likely arose through slipped-strand mispairing and replication slippage, known to contribute to the high variability of this region in insects (Ballard and Kreitman 1995). The observed hairpin and cruciform motifs, driven by high AT content, may support mtDNA replication and transcription, facilitating mitochondrial function under energy-demanding conditions like flight and blood feeding (Saito et al. 2005). In *Ae. albopictus*, the polymorphism and lineage-specific patterns of these repeat units suggest a potential utility in population genetics and phylogeography (Birungi and Munstermann 2002). Variation in tandem repeat structures across geographically distinct populations may reflect historical demographic processes such as founder effects, population bottlenecks, or recent colonization events – factors that have likely contributed to the global invasion success of this species (Porretta et al., 2012). These repeat expansions or contractions can act as molecular signatures of microevolutionary change, offering finer resolution than conventional barcoding genes such as COI or ND5.

Furthermore, the rapid evolution of tandem repeats may be driven by replication slippage or unequal recombination (Levinson and Gutman 1987), and may be under relaxed purifying selection or even subject to positive selection in certain ecological contexts (Stoneking et al. 1991). This may be particularly relevant for traits such as thermal tolerance, host preference, and insecticide resistance – traits

known to vary across *Ae. albopictus* populations. Therefore, the tandem repeat structures not only represent a non-coding evolutionary mechanism but may also contribute to the ecological success and adaptive potential of *Ae. albopictus*. This therefore, underscores the importance of including such rapidly evolving non-coding regions in mitogenomic studies aimed at resolving population structure, evolutionary history, and vectorial capacity. Future studies incorporating tandem repeat diversity alongside whole-genome SNP data could help unravel the adaptive landscape of this globally significant mosquito species.

In contrast, the *Cx. p. molestus* CR is shorter, at 780 bp, and lacks tandem repeats (Figure S3), but possesses high AT content (88.6%), consistent with other Culex species. Although tandem repeats are absent, microsatellite-like sequences or AT-rich motifs may contribute regulatory functionality (Tikochinski et al. 2012). These architectural distinctions may represent lineage-specific adaptations to different ecological niches or reproductive strategies. Comparative analyses also confirmed a conserved CR architecture within the *Culex pipiens* complex, which includes morphologically indistinct but ecologically divergent forms such as *pipiens*, *molestus*, *pallens*, and *quinquefasciatus* (Vinogradova 2000; Harbach 2012).

This study validates the mitogenome utility for phylogenetic and population studies of mosquitoes, and it confirms the taxonomic status of *Ae. albopictus* and *Culex* species from South Korea. Likewise, we revealed through this study the mitogenome conservation across geographically distinct populations of *A. albopictus* and suggested possible hidden genetic divergence within the *Cx. p. molestus* form, particularly between Korean and Australian samples. This research not only enhances our understanding of the genetic foundations of disease transmission but also provides a foundation for the development of targeted interventions and control strategies.

Furthermore, the divergence time analysis highlights key evolutionary timelines that may underline differences in vector competence, ecological specialization, and adaptation strategies among *Aedes*, *Culex*, and *Anopheles* mosquitoes. The placement of *Ae. albopictus* and *Cx. p. molestus* on distinct evolutionary branches with temporally separated divergence points reflects their independent evolutionary trajectories despite their shared role as arbovirus vectors. This research has practical implications for vector surveillance, disease risk assessment, and control strategies, particularly in regions experiencing rapid ecological change and globalization-driven mosquito dispersal. Through the integration of genomic knowledge into targeted interventions, we move closer to a future where effective strategies can alleviate the burden of these diseases and safeguard the health of vulnerable populations.

## Disclosure statement

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

## Funding

This research was funded by the Basic Science Research Program through the National Research Foundation of Korea (NRF), supported by the Ministry of Education [Grant Number RS-2024-00461502]. This work was supported by the National Research Foundation of Korea (NRF) grant to UWH funded by the Korea government (MSIT) [Grant Number RS-2025-00561309].

## Data availability statement

All mitochondrial genome sequences produced in this study have been submitted to the NCBI database under the accession numbers PQ584793 (*Aedes albopictus*) and PQ584792 (*Culex pipiens molestus*).

## References

Achilli A et al. 2008. Mitochondrial genomes of extinct aurochs survive in domestic cattle. Curr Biol. 18(4):R157–R158. https://doi.org/10.1016/j.cub.2008.01.019

Achilli A et al. 2012. Mitochondrial genomes from modern horses reveal the major haplogroups that underwent domestication. Proc Natl Acad Sci USA. 109(7):2449–2454. https://doi.org/10.1073/pnas.1111637109

Akintola AA, Kim G, Adeniyi KA, Hwang UW. 2024. Prevalence of *Wolbachia* endosymbiont in *Culex molestus* mosquitoes from South Korea. Entomol Res. 54(1):e12696. https://doi.org/10.1111/1748-5967.12696

Akintola AA, Park B, Choi EH, Hwang UW. 2022. Complete mitochondrial genome of a malaria vector mosquito *Anopheles sinensis* from South Korea. Mitochondrial DNA Part B. 7(5):881–883. https://doi.org/10.1080/23802359.2022.2077665
Avise JC. 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Conserv Biol. 9(3):686–690. https://doi.org/10.1046/j.1523-1739.1995.09030686.x
Ballard JW, Kreitman M. 1995. Is mitochondrial DNA a strictly neutral marker? Trends Ecol Evol. 10(12):485–488. https://doi.org/10.1016/S0169-5347(00)89195-8
Battaglia V et al. 2016. The worldwide spread of the tiger mosquito as revealed by mitogenome haplogroup diversity. Front Genet. 7:208. https://doi.org/10.3389/fgene.2016.00208
Beebe NW et al. 2013. Tracing the tiger: Population genetics provides valuable insights into the *Aedes (Stegomyia) albopictus* invasion of the Australasian region. PLoS Negl Trop Dis. 7(8):e2361. https://doi.org/10.1371/journal.pntd.0002361
Benedict MQ, Levine RS, Hawley WA, Lounibos LP. 2007. Spread of the tiger: global risk of invasion by the mosquito Aedes albopictus. Vector-Borne Zoonotic Dis. 7(1):76–85. https://doi.org/10.1089/vbz.2006.0562
Benson G. 1999. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 27(2):573–580. https://doi.org/10.1093/nar/27.2.573
Bernt M et al. 2013b. MITOS: Improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2):313–319. https://doi.org/10.1016/j.ympev.2012.08.023
Bernt M, Braband A, Schierwater B, Stadler PF. 2013a. Genetic aspects of mitochondrial genome evolution. Mol Phylogenet Evol. 69(2):328–338. https://doi.org/10.1016/j.ympev.2012.10.020
Bhatt S et al. 2013. The global distribution and burden of dengue. Nature. 496(7446):504–507. https://doi.org/10.1038/nature12060
Birungi J, Munstermann LE. 2002. Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial ND5 sequences: evidence for an independent invasion into Brazil and United States. Ann Entomol Soc Am. 95(1):125–132. https://doi.org/10.1603/0013-8746(2002)095[0125:GSOAAD]2.0.CO;2
Bonizzoni M, Gasperi G, Chen X, James AA. 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. Trends Parasitol. 29(9):460–468. https://doi.org/10.1016/j.pt.2013.07.003
Boore JL. 1999. Animal mitochondrial genomes. Nucleic Acids Res. 27(8):1767–1780. https://doi.org/10.1093/nar/27.8.1767
Bouckaert R et al. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Comput Biol. 15(4):e1006650. https://doi.org/10.1371/journal.pcbi.1006650
Cameron SL. 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. Annu Rev Entomol. 59(1):95–117. https://doi.org/10.1146/annurev-ento-011613-162007
Chan PP, Lowe TM. 2019. tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences. In M. Kollmar. (Eds.), Gene prediction. Methods in molecular biology, vol 1962. Humana, New York, NY. https://doi.org/10.1007/978-1-4939-9173-0_1
Choi EH et al. 2020. The complete mitochondrial genome of *Plumarella spinosa* (Octocorallia: Calcaxonia: Primnoidae) from South Korea. Mitochondrial DNA Part B. 5(1):725–726. https://doi.org/10.1080/23802359.2020.1715275
Choi EH et al. 2021. The mitochondrial genome of a giant water bug *Lethocerus deyrollei* (Hemiptera: Belostomatidae) from South Korea. Mitochondrial DNA Part B. 6(3):1001–1003. https://doi.org/10.1080/23802359.2021.1893616
Choi EH, Hwang UW. 2023. Complete mitochondrial genome of a golden orb-web spider *Trichonephila clavata* (Chelicerata, Arachnida) from South Korea. Mitochondrial DNA Part B. 8(7):723–725. https://doi.org/10.1080/23802359.2021.1955633
Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: More models, new heuristics and parallel computing. Nat Methods. 9(8):772. https://doi.org/10.1038/nmeth.2109
do Nascimento BLS et al. 2021. First description of the mitogenome and phylogeny of *Culicinae* species from the Amazon region. Genes (Basel). 12(12):1983. https://doi.org/10.3390/genes12121983
Eskildsen GA et al. 2018. Maternal invasion history of *Aedes aegypti* and *Aedes albopictus* into the Isthmus of Panama: Implications for the control of emergent viral disease agents. PLoS One. 13(3):e0194874. https://doi.org/10.1371/journal.pone.0194874
Fall G, Loucoubar C, Diallo M, Sall AA, Faye O. 2014. Vector competence of *Culex neavei* and *Culex quinquefasciatus* (Diptera: Culicidae) from Senegal for lineages 1, 2, Koutango, and a putative new lineage of West Nile virus. Am Soc Trop Med Hyg. 90(4):747–754. https://doi.org/10.4269/ajtmh.13-0405
Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. 2011. "Bird-biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect Genet Evol. 11(7):1577–1585. https://doi.org/10.1016/j.meegid.2011.08.013
Fonseca DM et al. 2004. Emerging vectors in the Culex pipiens complex. Science. 303(5663):1535–1538. https://doi.org/10.1126/science.1094247
Guillemaud T, Pasteur N, Rousset F. 1997. Contrasting levels of variability between cytoplasmic genomes and incompatibility types in the mosquito *Culex pipiens*. Proc R Soc Lond Ser B Biol Sci. 264(1379):245–251. https://doi.org/10.1098/rspb.1997.0035
Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 41:95–98.

Harbach RE. 2012. *Culex pipiens*: Species versus species complex – taxonomic history and perspective. J Am Mosq Control Assoc. 28(Suppl. 4):10–23. https://doi.org/10.2987/8756-971X-28.4.10
Harrison RG. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. Trends Ecol Evol. 4(1):6–11. https://doi.org/10.1016/0169-5347(89)90006-2
Hwang UW, Kim W. 1999. General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics. Korean J Parasitol. 37(4):215–228. https://doi.org/10.3347/kjp.1999.37.4.215
Kilpatrick AM et al. 2005. West Nile virus risk assessment and the bridge vector paradigm. Emerg Infect Dis. 11(3):425–429. https://doi.org/10.3201/eid1103.040364
Kim G et al. 2019. The complete mitochondrial genome of an Asian crested ibis *Nipponia nippon* (Pelecaniformes, Threskiornithidae) from South Korea. Mitochondrial DNA Part B. 4(2):3707–3708. https://doi.org/10.1080/23802359.2019.1680321
Kim IH et al. 2024. Complete mitochondrial genome of *Acanthochitona defilippii* (Polyplacophora: Chitonida) from South Korea. Mitochondrial DNA Part B. 9(8):1029–1033. https://doi.org/10.1080/23802359.2024.2386403
Knight KL. 1978. Supplement to the catalog of the mosquitoes of the world (Diptera: Culicidae). Entomological Society of America.
Kotsakiozi P et al. 2017. Population genomics of the Asian tiger mosquito, *Aedes albopictus*: insights into the recent worldwide invasion. Ecol Evol. 7(23):10143–10157. https://doi.org/10.1002/ece3.3514
Krzywinski J et al. 2011. Analysis of the evolutionary forces shaping mitochondrial genomes of a Neotropical malaria vector complex. Mol Phylogenet Evol. 58(3):469–477. https://doi.org/10.1016/j.ympev.2011.01.003
Larkin MA et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics. 23(21):2947–2948. https://doi.org/10.1093/bioinformatics/btm404
Laslett D, Canbäck B. 2008. ARWEN, a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics. 24(2):172–175. https://doi.org/10.1093/bioinformatics/btm573
Levinson G, Gutman GA. 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol. 4(3):203–221.
Li M et al. 2022. Mitochondrial phylogenomics provides insights into the phylogeny and evolution of spiders (Arthropoda: Araneae). Zool Res. 43(4):566–584. https://doi.org/10.24272/j.issn.2095-8137.2021.418
Medlock JM et al. 2012. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector-Borne Zoonotic Dis. 12(6):435–447. https://doi.org/10.1089/vbz.2011.0814
Osellame LD, Blacker TS, Duchen MR. 2012. Cellular and molecular mechanisms of mitochondrial function. Best Pract Res Clin Endocrinol Metab. 26(6):711–723. https://doi.org/10.1016/j.beem.2012.05.003
Park B et al. 2021. The complete mitochondrial genome of the two-spotted cricket *Gryllus bimaculatus* (Orthoptera: Gryllidae) from South Korea. Mitochondrial DNA Part B. 6(3):1144–1146. https://doi.org/10.1080/23802359.2021.1901617
Park B, Hwang UW. 2022. The complete mitochondrial genome of the woodwasp *Euxiphydria potanini* (Hymenoptera, Xiphydrioidea) and phylogenetic implications for symphytans. Sci Rep. 12(1):17677. https://doi.org/10.1038/s41598-022-21457-0
Porretta D et al. 2012. Glacial History of a Modern Invader: Phylogeography and Species Distribution Modelling of the Asian Tiger Mosquito Aedes albopictus. PLoS ONE. 7(9):e44515.
Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol. 67(5):901–904. https://doi.org/10.1093/sysbio/syy032
Reidenbach KR et al. 2009. Phylogenetic analysis and temporal diversification of mosquitoes (Diptera: Culicidae) based on nuclear genes and morphology. BMC Evol Biol. 9(1):298. https://doi.org/10.1186/1471-2148-9-298
Reinert JF. 2000. Revised list of currently recognized species in the genus Aedes (Diptera: Culicidae). J Am Mosq Control Assoc. 16(3):175–188.
Reinert JF, Harbach HR, Kitching JI. 2004. Phylogeny and classification of *Aedini* (Diptera: Culicidae), based on morphological characters of all life stages. Zool J Linn Soc. 142(3):289–368. https://doi.org/10.1111/j.1096-3642.2004.00144.x
Reinert JF, Harbach RE, Kitching IJ. 2009. Phylogeny and classification of tribe *Aedini* (Diptera: Culicidae). Zool J Linn Soc. 157(4):700–794. https://doi.org/10.1111/j.1096-3642.2009.00570.x
Rueda LM. 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. Zootaxa. 589(1):1–60. https://doi.org/10.11646/zootaxa.589.1.1
Russell RC. 2012. A review of the status and significance of the species within the *Culex pipiens* group in Australia. J Am Mosq Control Assoc. 28(Suppl. 4):24–27. https://doi.org/10.2987/8756-971X-28.4s.24
Ryu J, Choi KS. 2022. Species diversity of the *Culex pipiens* complex in the Republic of Korea. Entomol Res. 52(8):376–381. https://doi.org/10.1111/1748-5967.12610
Saito S, Tamura K, Aotsuka T. 2005. Replication origin of mitochondrial DNA in insects. Genetics. 171(4):1695–1705. https://doi.org/10.1534/genetics.105.046243
Schaffner F, Medlock JM, Van Bortel W. 2013. Public health significance of invasive mosquitoes in Europe. Clin Microbiol Infect. 19(8):685–692. https://doi.org/10.1111/1469-0691.12189
Shaikevich EV, Vinogradova EB, Ivanitsky AV. 2016. Molecular and ecological characteristics of *Culex pipiens* s.l. in Russia. Parasit Vectors. 9:639.

Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT. 2006. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annu Rev Ecol Evol Syst. 37(1):545–579. https://doi.org/10.1146/annurev.ecolsys.37.091305.110018

Smith JL, Fonseca DM. 2004. Rapid assays for identification of members of the *Culex* (*Culex pipiens*) complex, their hybrids, and other sibling species (Diptera: Culicidae). Am J Trop Med Hyg. 70(4):339–345. https://doi.org/10.4269/ajtmh.2004.70.339

Stoneking M, Hedgecock D, Higuchi RG, Vigilant L, Erlich HA. 1991. Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence-specific oligonucleotide probes. Am J Hum Genet. 48(2):370–382.

Tao M et al. 2014. Animal mitochondria: Evolution, function, and disease. Curr Mol Med. 14(1):115–124. https://doi.org/10.2174/1566524013136660081

Tikochinski Y et al. 2012. Mitochondrial DNA STR analysis as a tool for studying the green sea turtle (*Chelonia mydas*) populations: the Mediterranean Sea case study. Mar Genomics. 6:17–24. https://doi.org/10.1016/j.margen.2012.01.002

Torroni A, Achilli A, Macaulay V, Richards M, Bandelt HJ. 2006. Harvesting the fruit of the human mtDNA tree. Trends Genet. 22(6):339–345. https://doi.org/10.1016/j.tig.2006.04.001

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44(W1):W232–W235. https://doi.org/10.1093/nar/gkw256

Vinogradova EB. 2000. *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pensoft Publishers.

Wiegmann BM et al. 2011. Episodic radiations in the fly tree of life. Proc Natl Acad Sci USA. 108(14):5690–5695. https://doi.org/10.1073/pnas.1012675108

Yang Z. 2007. PAML 4: Phylogenetic analysis by maximum likelihood. Mol Biol Evol. 24(8):1586–1591. https://doi.org/10.1093/molbev/msm088

Yang Z, Nielsen R. 2000. Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. Mol Biol Evol. 17(1):32–43. https://doi.org/10.1093/oxfordjournals.molbev.a026236

Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science. 292(5517):686–693. https://doi.org/10.1126/science.1059412

Ze-Ze L et al. 2020. Mitogenome diversity of *Aedes (Stegomyia) albopictus*: Detection of multiple introduction events in Portugal. PLoS Negl Trop Dis. 14(9):e0008657. https://doi.org/10.1371/journal.pntd.0008657

Zhang NX, Zhang YJ, Yu G, Chen B. 2013. Structure characteristics of the mitochondrial genomes of Diptera and design and application of universal primers for their sequencing. Acta Entomol Sin. 56:398–407.

Zhong D et al. 2013. Genetic analysis of invasive Aedes albopictus populations in Los Angeles County, California, and its potential public health impact. PLoS One. 8(7):e68586. https://doi.org/10.1371/journal.pone.0068586

Žitko T, Kovačić A, Desdevises Y, Puizina J. 2011. Genetic variation in East-Adriatic populations of the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), inferred from NADH5 and COI sequence variability. Eur J Entomol. 108(4):501–508. https://doi.org/10.14411/eje.2011.065