

Article

Sequencing and Analysis of the Mitochondrial Genome of *Aedes aegypti* (Diptera: Culicidae) from the Brazilian Amazon Region

Andreolina Alves de Sousa ¹, Ana Cecília Ribeiro Cruz ^{2,3}, Fábio Silva da Silva ^{2,3}, Sandro Patroca da Silva ², Joaquim Pinto Nunes Neto ^{2,3}, Maria Claudene Barros ⁴, Elmary da Costa Fraga ⁴ and Iracilda Sampaio ^{5,*}

- ¹ Post-Graduate Program in Genetics and Molecular Biology, Federal University of Pará, Belém 66075-110, Pará, Brazil; andrelbio@yahoo.com.br
- ² Evandro Chagas Institute (IEC/SVS/MS), Department of Arbovirology and Hemorrhagic Fevers, Ananindeua 67030-000, Pará, Brazil; anacecilia@iec.gov.br (A.C.R.C.); fabiodasilva@iec.gov.br (F.S.d.S.); sandrosilva@iec.gov.br (S.P.d.S.); joaquimneto@iec.gov.br (J.P.N.N.)
- ³ Post-Graduate Program in Parasite Biology in the Amazon, Center of Biological and Health Sciences, Pará State University, Belém 66095-662, Pará, Brazil
- ⁴ Laboratory of Genetics and Molecular Biology (GENBIMOL), Maranhão State University, Caxias 65604-380, Maranhão, Brazil; mbdene@yahoo.com.br (M.C.B.); elmaryfraga@yahoo.com.br (E.d.C.F.)
- ⁵ Laboratory of Evolution, Institute of Coastal Studies, Federal University of Pará, Bragança 68600-000, Pará, Brazil
- * Correspondence: ira@ufpa.br

Simple Summary: Responsible for the transmission of arboviruses, the mosquito *Aedes aegypti* is a major challenge to public health in Brazil, particularly in urban areas located within tropical and subtropical regions. The recent epidemiological bulletins from the Brazilian Health Ministry in the state of Maranhão have reported high levels of urban infestation by this vector, which represents a potential risk for the occurrence of new epidemics. In this context, the present study describes the partial mitochondrial genome of a sample of *Ae. aegypti* from the Brazilian state of Maranhão. Studies of this type provide important insights into the biological evolution of this important species and have the potential to contribute to the development of increasingly effective strategies for the control of this disease vector.



Citation: Sousa, A.A.d.; Cruz, A.C.R.; Silva, F.S.d.; Silva, S.P.d.; Neto, J.P.N.; Barros, M.C.; Fraga, E.d.C.; Sampaio, I. Sequencing and Analysis of the Mitochondrial Genome of *Aedes aegypti* (Diptera: Culicidae) from the Brazilian Amazon Region. *Insects* **2023**, *14*, 938. <https://doi.org/10.3390/insects14120938>

Received: 19 October 2023

Accepted: 3 November 2023

Published: 11 December 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: *Aedes aegypti* is a mosquito native to the African continent, which is now widespread in the tropical and subtropical regions of the world. In many regions, it represents a major challenge to public health, given its role in the cycle of transmission of important arboviruses, such as Dengue, Zika, and Chikungunya. Considering the epidemiological importance of *Ae. aegypti*, the present study sequenced the partial mitochondrial genome of a sample collected in the municipality of Balsas, in the Brazilian state of Maranhão, followed by High Throughput Sequencing and phylogenetic analyses. The mitochondrial sequence obtained here was 15,863 bp long, and contained 37 functional subunits (thirteen PGCs, twenty-two tRNAs and two rRNAs) in addition to a partial final portion rich in A+T. The data obtained here contribute to the enrichment of our knowledge of the taxonomy and evolutionary biology of this prominent disease vector. These findings represent an important advancement in the understanding of the characteristics of the populations of northeastern Brazil and provide valuable insights into the taxonomy and evolutionary biology of this prominent disease vector.

Keywords: Legal Amazon; Culicidae; mtDNA; phylogeny; population genetics

1. Introduction

Mosquitos (Diptera: Culicidae) have a virtually cosmopolitan distribution, particularly in the world's temperate and tropical regions [1]. While the first mosquito species were

described in the eighteenth century, the link between these insects and the transmission of infectious diseases, such as filariasis and malaria, was only discovered at the end of the nineteenth century. Almost two centuries after the first scientific studies of these organisms, approximately 3600 mosquito species have now been described formally, although many more species are assumed to exist, highlighting the need for extensive revision of the Culicidae and, in particular, studies that provide insights into the evolutionary drivers of the group [1].

In epidemiological terms, *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) is one of the most important mosquito species, not least because of its intimate relationship with human populations, which has allowed it to spread throughout all the world's continents, except Antarctica. The species is often targeted specifically in government campaigns for the control of disease vectors and is often associated with human migrations and unregulated urban development [2]. This mosquito is involved in the transmission cycle of a large number of viruses, including the principal arboviruses that affect human populations [3], that is, *Orthoflavivirus denguei* (DENV serotypes I, II, III and IV), *Orthoflavivirus flavi* (YFV), *Orthoflavivirus zikaense* (ZIKV), and *Chikungunya virus* (CHIKV).

Aedes aegypti has a complex evolutionary history, with studies indicating an initial major dispersal from Africa to the Americas, and, subsequently, to Asia, although there is also evidence of the parallel dispersal of two distinct lineages that originated in eastern and western Africa [4]. The species was almost certainly introduced into the Americas during the Age of Discovery, between the sixteenth and eighteenth centuries, when it expanded rapidly throughout most of tropical South and Central America [5,6].

In recent decades, a number of studies have investigated the genetic variability of *Ae. aegypti* populations, mainly using molecular markers extracted from regions of the nuclear [7,8] and mitochondrial genomes [5,9–12]. These studies have revealed significant differences among populations, which have led to the hypothesis that two principal *Ae. aegypti* lineages are found in Brazil, and worldwide. In this context, the mitochondrial (mtDNA) genome is an important tool for evolutionary studies, given its uniparental inheritance, lack of recombination and high mutation rates, in comparison with the nuclear genome [13–16]. Mitochondrial markers are especially useful for the study of vectors of medical and epidemiological importance [17–25].

The state of Maranhão is located in the northeast region of Brazil, and coincides with the transition zone between the Amazon, Cerrado and Caatinga biomes. Recent epidemiological bulletins of the Brazilian Health Ministry, Maranhão have revealed high levels of urban infestation by *Ae. aegypti*, which represents a potential risk for public health. Given the epidemiological importance of this vector and its high population densities in the state, it is necessary to understand the genetic variability of *Ae. aegypti* and its population structure, in order to support the development of effective strategies of population control, adapted to the characteristics of the populations found in northeastern Brazil.

Given the global epidemiological importance of this species, the present study provides the first description of the mitochondrial genome of a representative sample of *Ae. aegypti* population from the state of Maranhão, in northeastern Brazil, using High Throughput Sequencing for the first time.

2. Materials and Methods

2.1. Collection of the Biological Samples and the Extraction of the Total DNA

The biological samples of *Ae. aegypti* analysed in the present study were collected during expeditions to the municipality of Balsas, in the state of Maranhão (−7.53°29.17' S, −46.03°74.56' W), in the mid-north region of Brazil (Figure 1). This region is a subdivision of the Brazilian Northeast, which coincides with the transition zone between the Amazon and Cerrado biomes, and also includes the western half of the state of Piauí [26]. The samples were collected using 30 ovitraps, which were distributed in different neighbourhoods of the municipality at intervals of at least 1 km. The traps were set in shady and poorly lit areas protected from the rain in the vicinity of residential infrastructure. The sites were

selected previously during the rapid index surveys of *Ae. aegypti* (LIRAA) conducted in the municipality. The traps were retrieved after six days and transported to the Genetics and Molecular Biology Laboratory at the Caxias campus of Maranhão State University (UEMA).

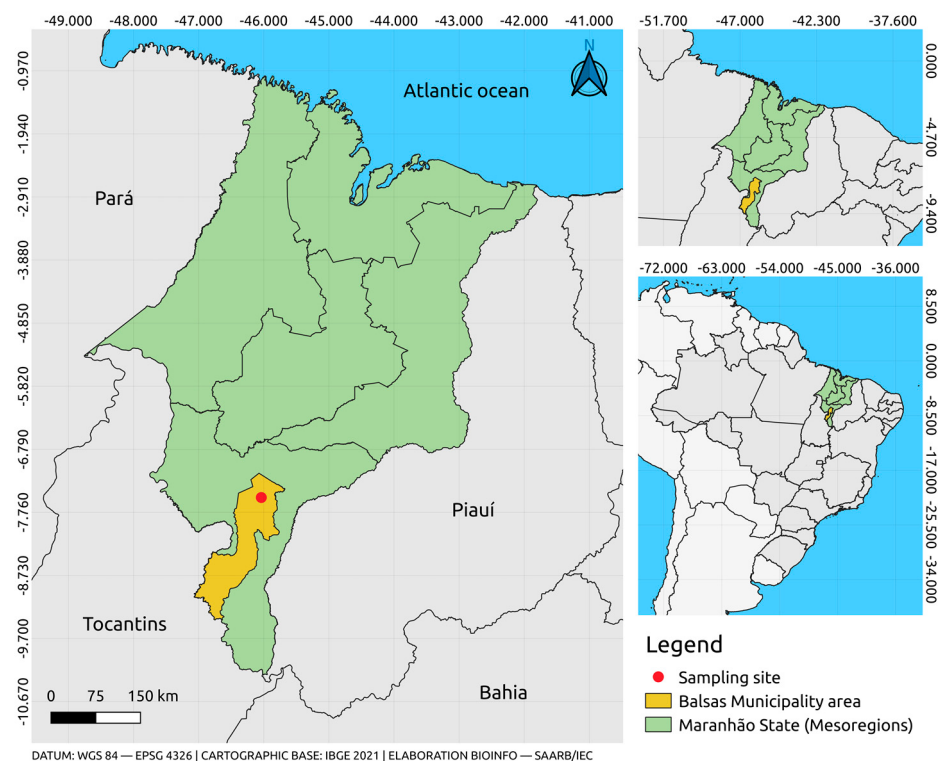


Figure 1. Location of the municipality of Balsas, in Maranhão state, showing the site from which the biological samples were collected.

The ovitraps contained small (12 cm × 2.8 cm) palettes on which the mosquitos laid their eggs during the trapping period. In the laboratory, the palettes were immersed in water to allow for the eclosion of the larvae, which were then kept in artificial nurseries until the emergence of the adults, which were identified using stereoscopic loupes and the dichotomous classification key, developed by Consoli and Lourenço-de-Oliveira [27], which is based on specific features of the external morphology of the adult specimens. Adults of the study species (*Ae. aegypti*) were transferred to entomological cages designed for breeding, where they were maintained with a 10% saccharose solution.

After two feeding cycles, a total of 20 females were isolated to lay eggs, which were isolated in individual artificial incubators until the eclosion of the larvae, which were fed until the fourth development stage (L4), following the protocol established by Santos et al. [28]. Once the larvae reached stage L4, they were transferred to 1.5 mL Eppendorf micro-tubes containing 70% alcohol as a preserver and stored in a freezer at −80 °C until the extraction of the total DNA.

The total DNA of each sample was extracted using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA), following the manufacturer's recommendations. The products extracted from each sample were quantified using a Qubit 2.0 fluorometer (Invitrogen, Waltham, MA, USA), together with a dsDNA Hs Assay kit (Invitrogen, Waltham, MA, USA).

2.2. Genomic Sequencing

The genomic sequencing, computer analyses and compilation of the results were all conducted at the Arbovirology and Hemorrhagic Fevers Section of the Evandro Chagas Institute (SAARB-IEC/MS/SVSA) in Ananindeua. The genomic library was compiled from a single sample, which had been quantified previously and standardised to a concentration

of 0.2 ng/μL, and then fragmented and marked with two adapter sequences (i7 and i5) using the Nextera XT DNA Library Preparation kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol. The sample was then quantified using a Qubit 2.0 fluorometer (Invitrogen) and the fragment size was evaluated using a High Sensitivity DNA analysis kit (Agilent Technologies, Santa Clara, CA, USA) in a BioAnalyzer (Agilent Technologies). The final product was used for genomic sequencing using a NextSeq 500/550 High Output kit (Illumina) for 300 cycles (2 × 150) on a NextSeq 500 System platform.

2.3. Data Processing and Description of the Genome

The raw reads were initially evaluated qualitatively using Fastp v. 0.23.2 [29], configured to remove the adapter sequences, reads with a PHRED quality score of less than 20, and reads of less than 50 nt in length. To better isolate the reads that correspond to those of the study species, the data were mapped against a reference genome (*Aedes aegypti* L5.0, mtDNA GenBank ID: NC_035159) using a combination of Bowtie2 v.2.5.1 [30] and Samtools v.1.17 [31]. The filtered data were then used to mount the genome using the De Novo method. The contig was assembled using the MEGAHIT v.1.2.9 software [32], with the default mounting configurations (in k-mer lengths of 21, 29, 39, 59, 79, 99, 119 and 141 nt). The mitochondrial contigs obtained here were identified using the DIAMOND v.2.1.6.160 [33] in BlastX alignment mode, considering an e-value of 10^{-5} , and inspected manually in Geneious v.11.1.5 [34].

The final mitochondrial sequence was annotated using the online MITOchondrial genome annotation Server tool (MITOS) [35] and fitted to a circular configuration, based on the identification of overlaps using Blastn v.2.14.0 [36], and was also used as a reference for the remapping of the mitochondrial reads in Bowtie2, to obtain their coverage metrics. The linear structure of the sequence was determined using CGview [37], and its composition and nucleotide parameters and the relative synonymous codon usage (RSCU) were obtained using MEGA X v.10.2.6 [38] and Geneious v.11.1.5 [34], respectively. Skews in the nucleotide composition were calculated using the formulas $AT\text{-skew} = (A - T)/(A + T)$ and $GC\text{-skew} = (G - C)/(G + C)$ [39]. To evaluate the selection pressure affecting the PCGs of the study species of the tribe Aedini, the ratios of non-synonymous (dN) to synonymous (dS) substitutions were calculated in CodeML, available in the PAML package [40]. All the graphs presented here were produced using the R software v.4.2.3 (available at: <https://www.r-project.org> accessed on 23 June 2023), together with the *ggplot2*, *reshape2* and *pheatmap* packages.

2.4. Phylogenetic Analysis

The phylogeny of the mosquitoes was reconstructed based on all 13 protein coding regions of the *Ae. aegypti* sequence obtained here, together with those of the other 28 mtDNAs available in the public databases (Supplementary Table S1). The regions corresponding to each PCG of the target sequences were extracted in parts, aligned using MAFFT v.7.520 [41], inspected manually in Aliview v.1.28 [42], and concatenated using Seqkit v.2.4.0 [43]. The nucleotide distances between the study taxa were obtained using MEGA X (based on the maximum likelihood composition model). The phylogeny was reconstructed using the maximum likelihood method, with the prior definition of the best nucleotide substitution model (GTR+F+I+G4) based on the Akaike information criterion (AIC), using the IQ-TREE v.1.6.12 software [44], with the bootstrapping (BPP) set to 1000 repetitions. The topology obtained from these analyses was visualized in FigTree v.1.4.4 (available at: <http://tree.bio.ed.ac.uk/software/figtree> accessed on 26 June 2023) and edited with Inkscape v.0.92 (available at: <https://inkscape.org/pt-br> accessed on 30 June 2023).

3. Results

The genomic sequencing generated a total of 75.5 million reads, with 94% approval, following the quality control, and after the exclusion of the adapters and the bases with

PHRED quality scores of less than 20. A contig of 15,863 bp was also obtained (Figure 2A,B) with a mean coverage depth of $217.4\times$, which corresponds to 28,539 of the total number of reads generated, being composed with 37 functional subunits (13 PCGs, 22 tRNAs and 2 rRNAs) with a partial portion of the control region rich in adenine and thymine (A+T) (Supplementary Table S2).

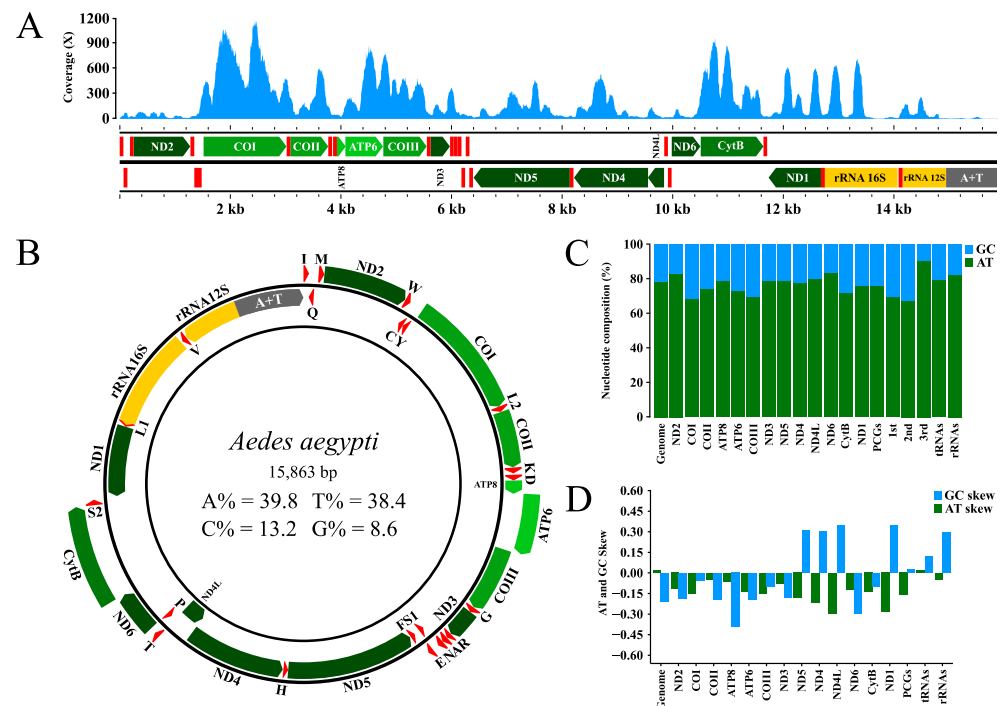


Figure 2. (A) Genomic coverage and structural organisation of the sequence obtained in the present study, showing the 37 functional subunits (13 PCGs, 22 tRNAs and 2 rRNAs), and the partial portion control region rich in adenine and thymine (A+T), (B) circular configuration of the genome, (C) AT% and GC% nucleotide composition and (D) AT/GC skews.

The overall AT content of the sequence obtained here is 78.2% (Figure 2C), with a generally positive AT skew (which indicates a greater adenine content in comparison with thymine) and a negative GC skew (indicating a relatively greater cytosine content versus guanine) (Supplementary Table S2), although with inversions, depending on the different arrangements of the subunits and regions evaluated (Figure 2D). An additional 22 small intergenic regions were also identified between the annotated subunits, ranging in length from 1 to 45 bp.

A typical secondary structure of the cloverleaf type was observed in 21 of the 22 tRNA subunits annotated here, which had lengths varying from 64 bp (tRNA^{Arg}) to 72 bp (tRNA^{Val}) with a mean AT content of 77.9% (Supplementary Table S2). This structure has four arms (amino acid acceptor (AA), dihydrouridine (DHU), TΨC and anticodon acceptor (AC)) and four loops (AA, DHU, TΨC and Variable (V)). The only variation was observed in the tRNA^{Ser1} subunit, in which the dihydrouridine (DHU) arm has been substituted by a DHU loop (Supplementary Figure S1).

The 13 PCGs annotated from the sequence obtained here vary in length from 159 (ATP8) to 1740 bp (ND5), with an AT content ranging from 72.5% (ND2) to 83.6% (CytB). The most frequent start codons were of two types (ATG and ATT), with complete stop codons of the TAA types. Excluding stop codons, a total of 3719 amino acid triplets were recorded, with a significant AT skew in the third position of the amino acids expressed in the codifying regions of the sequence. The relative synonymous codon usage (RSCU) indicates that only the triplet AGG (tRNA^{Ser1}) was not present in the PCGs, and that a majority of the codons terminating with adenine or uracil (thymine) were expressed signifi-

cantly ($RSCU > 1$) more often than those terminating with cytosine or guanine. Overall, the amino acid expressed most frequently in the sequence was UUA ($tRNA^{Leu1}$), with 484 occurrences ($RSCU = 4.93$), while the least expressed was ACG ($tRNA^{Tyr}$), with only a single occurrence, with $RSCU = 0.02$ (Figure 3A).

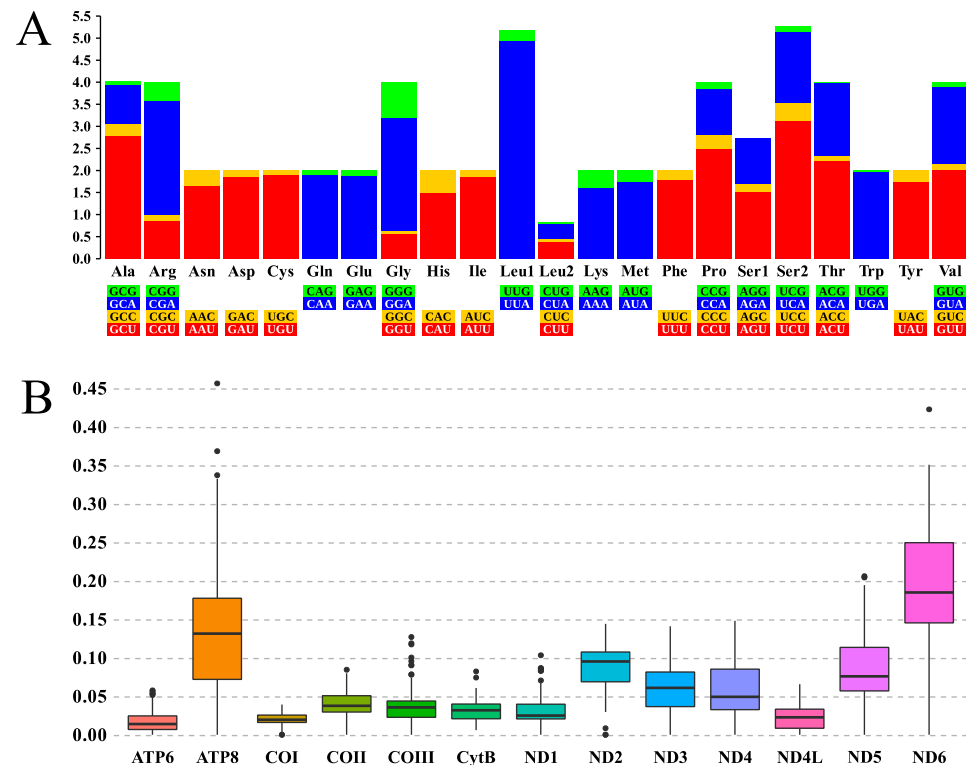


Figure 3. (A) Relative synonymous codon usage (RSCU) of the mtDNA of *Ae. aegypti*. The RSCU values are shown on the y axis, while the families of synonymous codons and their respective codifying triplets are shown on the x axis. (B) Boxplot of the mean evolutionary pressure that affects the PCGs identified in the present study, based on the ratio of non-synonymous (dN) to synonymous (dS) substitutions (dN/dS) recorded in the set of taxa closed related to the genus *Aedes*, including the sequence obtained here. The dN/dS ratios are shown on the y axis, and the PCGs, on the x axis.

The evaluation of the parameters of the evolutionary pressures that influence the PCGs was based on the ratio of non-synonymous to synonymous substitutions (dN/dS) and the comparison of the sequence obtained here with those of other taxa related closely to the genus *Aedes*. This analysis indicated that the different codifying regions have evolved globally under the influence of purifying pressure ($dN/dS > 1$), with mean ratios ranging from 0.001 ± 0.0586 in *ATP6* to 0.001 ± 0.4573 in *ATP8* (Figure 3B).

The sequence obtained in the present study was most similar to those of other *Ae. aegypti* specimens, in particular, sequences with the Genbank IDs MK575476 and NC_035159, with the latter used as a reference for mapping and annotating the DNA structure of the sample analysed here. Based on the analysis of nucleotide distances, the mean pairwise distance recorded among the 29 taxa used to reconstruct the phylogeny, including the sequence obtained here, was 0.09, with values ranging from 0.0005 to 0.1874 (Supplementary Table S3). In accordance with the results of the distance matrix, the phylogeny reconstructed by the maximum likelihood method identified a well-structured monophyletic group with two principal clades, which include representatives of the subfamilies Anophelinae and Culicinae (tribe Aedini), rooted externally by *Dixella aestivalis* (Figure 4). The Culicinae clade is formed four principal sub-clades (*Haemagogus*, *Ochlerotatus*, *Stegomyia* and *Psorophora*), with BPP = 100%, highlighting the taxa most closely related

to genus *Aedes* (mean distance of 0.1). The sequence obtained here aligned with the other *Ae. aegypti* (BPP = 100%), as part of the sub-clade of the subgenus *Stegomyia*.

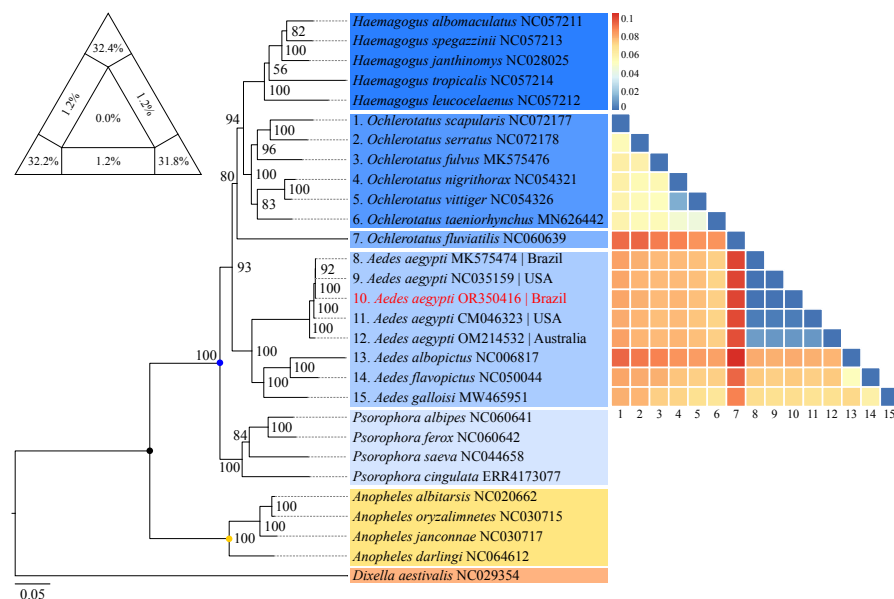


Figure 4. Reconstruction of the phylogeny using the maximum likelihood method of the mosquitoes analysed in the present study, based on the 13 concatenated PCGs of the *Ae. aegypti* obtained in this study and 28 other taxa available in public databases. Support values (BPP) are shown at each node. The coloured dots indicate the taxonomic groups formed in the analysis (the black dot indicates the clade of the Culicidae family; the yellow dot indicates the common ancestor of the Anophelinae subfamily; and the blue circle indicates the common ancestor of the Aedini tribe). The heat map on the right demonstrates the nucleotide distances, based on the maximum composite likelihood method, between taxa related to the genus *Aedes*, where the distances are color coded, ranging from most similar (blue) to most divergent (red). The triangle of values in the upper left corner presents a percentage representation of the quality of the phylogenetic signal for reconstructing the topology based on the set of sequences used. The sum of the sides (96.4%) indicates the percentage of high reliability topologies generated during the analysis.

4. Discussion

Ae. aegypti is now well established as one of the world's primary vectors of arboviruses, a role favoured by its highly synanthropic behaviour, its desiccation-resistant eggs and its ecological adaptability, which confers a degree of resistance to pesticides, and hamper the implementation of effective control measures [45–47]. These traits also contribute to the dispersal capacity of the species and its current worldwide distribution [48,49]. In the 1950s, the implementation of radical measures to control the species in Brazil and other South American countries led to its apparent eradication from the region until its reintroduction in 1970. Since this time, *Ae. aegypti* has become the disease vector with the greatest impact in the region for the transmission of the infectious agents responsible for diseases such as Dengue, Zika and Chikungunya, with thousands of cases being recorded annually, particularly in urban environments [50]. It is in these urban environments that the investigation of the genetic structure of the vector populations can contribute most to the development of the most effective strategies of control. These data not only contribute to the understanding of the adaptive capacity of the vector to different environments, in particular anthropogenic habitats, but also improve the scientific comprehension of the molecular interactions at the pathogen–host level [46,50,51].

The use of molecular markers in studies of the evolutionary biology of mosquitoes, in particular those derived from genomic regions such as the mitochondrial (mtDNA) and nuclear DNA (nDNA), have provided important insights into the evolutionary biology of these insects, as well as the genetic diversity of their populations, in particular in Brazil [46].

In this context, a series of previous studies have concluded that the *Ae. aegypti* populations of Brazil encompass two principal genetic groups, separated approximately by the transition zone at the limit of the Amazon forest [52–56]. The previous studies on populations from Maranhão, in particular, found high levels of intra-population differentiation, which were consistent with the coexistence of two *Ae. aegypti* genetic lineages in this state, which coincides with the ecological transition to the Amazon forest [8,11,57].

The present study describes the complete mitochondrial sequence of an *Ae. aegypti* specimen sampled from one of the populations found in the municipality of Balsas, in the Brazilian state of Maranhão. The sequence obtained is subdivided into 37 functional subunits, in addition to a partial portion of the control region rich in adenine and thymine (A+T), with the characteristic arrangement and disposition of the genes along the two strands of transcription, as observed in all the previous description of the mitochondrial genomes of mosquitoes, in particular, those of the tribe Aedini [20,22–24,58]. The nucleotide sequence obtained here share a high level of identity with a reference genome (GenBank ID: NC_035159), which was obtained in a study that also described the three chromosomes of the species [58].

In comparison with the reference genome, the sequence obtained in the present study had a general AT content higher than that of GC, with a positive global skew in AT and negative skew in GC, which is consistent with the larger proportions of adenine and cytosine found throughout the principal sequence. Despite these major similarities, the most obvious difference is the relative lack of polymorphisms, which are most concentrated between the *ND5* and *ND6* regions, in addition to a lack of coverage for the central part of the final portion of the sequence obtained here, which is rich in A+T. In metazoans, this region is associated with the replicative processes of the mtDNA, in particular, a large number of homopolymeric repetitions, considering the presence of significant variations in the length of this region due to the high nucleotide substitution and insertion/deletion rates, which result in clear differences in its length in the different culicid species [14,59,60]. However, in some of the studies that have described mitochondrial sequences by High Throughput Sequencing, certain difficulties arose in the sequencing of this region, possibly related to the problems associated with the recovery of the homopolymeric portions. Given this, the use of selective amplification methods for this region, such as conventional PCR and Sanger sequencing, have been considered to be a potentially valuable alternative, with satisfactory results in some similar studies [61–64].

The *Ae. aegypti* sequence obtained in the present study contained all 22 tRNAs predicted for the mtDNA, as observed in both other mosquitoes and other organisms, as well as their typical secondary structures, including the substitution of the dihydrouridine (DHU) arm by a DHU loop in tRNA^{Ser1} gene. With the exception of the 920 bp final portion, in fact, the sequence obtained here includes only 204 non-coding base pairs, divided among the 22 small intergenic regions, of which the largest was 45 bp, and was located between the tRNA^{Gln} and tRNA^{Met} genes, highlighting their compact structure. This has also been observed in other genomes of other mosquitoes, including a degree of overlap between some open read frames (ORFs), in particular those associated with regions involved in the codification of proteins, where, with the exception of only the *ND1* and *ND5* subunits, the start codons are all ATN, while all the stop codons are TAA, in addition to a high AT content in the third codon position in the expressed amino acid triplets. The considerable increase in the AT content at this position may be related to a less intense level of purifying selection against deleterious mutations in these regions [19,65].

In the specific case of the type of selection pressure affecting the PCGs of the sequence obtained in the present study, there was a clear global tendency for the purifying selection ($dN/dS < 1$), which was reflected in the much higher rates of synonymous substitutions, in comparison with non-synonymous substitutions, within the regions analysed. As observed in other studies, in addition, including those of the genus *Aedes* [22,24,65], the purifying selection was more intense in mitochondrial complexes III (cytochrome b) and IV (cytochrome oxidase c), in contrast with complex I (NADH) and the *ATP8* subunit,

in particular. While the mean dN/dS ratio recorded in the present study, considering all the taxa analysed, was relatively low in *ATP6*, the *COI* subunit presented the lowest proportions recorded in the present study, which reinforces the widespread use of this subunit as a molecular marker for the determination of phylogenetic relationships at a given taxonomic level [65–67], given its high rates of synonymous mutation in comparison with the regions of complexes I and V (in particular *ATP8*). While the dN/dS ratio of these regions is still below one, they have less conservative evolutionary restrictions.

Based on the whole concatenated sequence used for the phylogenetic reconstruction, the representatives of the tribe Aedini formed a well-structured monophyletic group that included five sub-clades, of which, two correspond to the genera *Haemagogus* and *Psorophora*, while the other three contain taxa closely related to the genus *Aedes*. It is important to note here that both the arrangement of the taxa and the classification of some of the *Aedes* subgenera have been the subject of intense revision and constant debate, particularly in relation to certain features of the external morphology. In this context, taxa previously considered to be subgenera of *Aedes*, such as *Ochlerotatus* and *Stegomyia*, have been treated as independent groups within the tribe Aedini by some authors, based on the polyphyletic relationships found by studies such as those of Reinert [68] and Reinert et al. [69,70]. However, these reclassifications are not supported universally, given that other studies [71] have not found sufficient support for the independence of the lineages.

The sequence obtained here was most similar to those of the other *Ae. aegypti*, with the clear formation of a sub-clade in *Stegomyia*. The relationships observed here among the *Aedes* sub-clades, considering *Ochlerotatus* and *Stegomyia*, together with *Oc. fluviatilis* (which was classified here as *Georgecraigius fluviatilis* in accordance with the proposal of Reinert [68]), are consistent with the polyphyletic arrangement proposed by this author based on morphological features. These findings also align with those of other studies based on the application of molecular markers to the taxonomy of the tribe Aedini [23,24,72,73].

Three thousand cases of Dengue were recorded in the state of Maranhão in the first half of 2023 alone, with an increase of 30% in the number of probable cases in comparison with the same period of the previous year [74]. This preoccupying trend may be related in particular to the intrinsic deficiencies of the measures adopted for the control and eradication of the populations of *Ae. aegypti*, the principal vector found in urban environments. In this context, it will be necessary for local research groups to evaluate the presence of different lineages of the species, associating this information with the mechanisms of dispersal of the mosquito and the spatial distribution of the outbreaks of Dengue in the state. The first genomic studies in the state of Maranhão focused on the use of mitochondrial (especially *ND4*) and nuclear (microsatellites) molecular markers to describe the genetic structuring of local populations [8,11,12]. Until recently, however, *High Throughput Sequencing* had not been used to determine these genomic features. In this case, through the definition of the complete mitochondrial sequence of *Ae. aegypti*, the present study intended to contribute to the understanding of the evolutionary dynamics of the species, as well as providing resources for its more reliable taxonomic identification, based on molecular features. These advances represent valuable insights for the establishment of guidelines for the development of innovative strategies for the control of this important disease vector.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/insects14120938/s1>, Figure S1—Secondary structures of tRNAs and rRNAs of *Aedes aegypti* OR350416; Table S1—Taxa used in the evolutionary analyses; Table S2—*Aedes aegypti* OR350416 nucleotide composition metrics; Table S3—Nucleotide distances by maximum likelihood composition.

Author Contributions: Conceptualisation, A.A.d.S., F.S.d.S. and A.C.R.C.; methods, A.A.d.S., F.S.d.S. and S.P.d.S.; validation, F.S.d.S., S.P.d.S. and A.C.R.C.; formal analysis, A.A.d.S., F.S.d.S. and S.P.d.S.; investigation, A.A.d.S. and F.S.d.S.; data curation, F.S.d.S. and S.P.d.S.; writing—original draft, A.A.d.S. and F.S.d.S.; writing—review and editing, A.A.d.S., F.S.d.S., S.P.d.S., M.C.B., J.P.N.N., E.d.C.F.,

A.C.R.C. and I.S.; visualisation, F.S.d.S., S.P.d.S. and A.C.R.C.; resources, J.P.N.N., A.C.R.C. and I.S.; supervision, M.C.B., E.d.C.F., A.C.R.C. and I.S.; project administration, M.C.B., E.d.C.F., A.C.R.C. and I.S.; funding acquisition, M.C.B., E.d.C.F., A.C.R.C. and I.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Programme for Academic Cooperation in Amazonia (public notice number 21/2018 PROCAD/AMAZONIA), process number 23038.005350/2018-78 and benefited from funding provided by the Brazilian Coordination for Higher Education Personnel Training (CAPES), grant number 88887.761304/2022-00, and the National Council for Scientific and Technological Development (CNPq), grant numbers 314522/2021-2 to A.C.R.C. and 309916/2021-6 to I.S. This study was supported by the Maranhão State Foundation for Research and Scientific and Technological Development (FAPEMA: public notice number: 002/2019–Universal) through the acquisition of laboratory infrastructure. Publication of the manuscript was financed by PROPESP/UFGA (PAPQ).

Data Availability Statement: All the data obtained during this study are available in the tables and figures included in the text, and in the Supplementary Material. The mitochondrial sequence obtained here was deposited in the GenBank database under accession code OR350416.

Acknowledgments: Brazilian Coordination for Higher Education Personnel Training (CAPES) for providing a doctoral scholarship, process number 88887.639719/2021-00.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Harbach, R. The Culicidae (Diptera): A Review of Taxonomy, Classification and Phylogeny. *Zootaxa* **2007**, *1668*, 591–638. [\[CrossRef\]](#)
2. Escobar, D.; Ortiz, B.; Urrutia, O.; Fontecha, G. Genetic Diversity among Four Populations of *Aedes aegypti* (Diptera: Culicidae) from Honduras as Revealed by Mitochondrial DNA Cytochrome Oxidase I. *Pathogens* **2022**, *11*, 620. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Espinal, M.A.; Andrus, J.K.; Jauregui, B.; Waterman, S.H.; Morens, D.M.; Santos, J.I.; Horstick, O.; Francis, L.A.; Olson, D. Emerging and Reemerging *Aedes*-Transmitted Arbovirus Infections in the Region of the Americas: Implications for Health Policy. *Am. J. Public Health* **2019**, *109*, 387–392. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Joyce, A.L.; Torres, M.M.; Torres, R.; Moreno, M. Genetic Variability of the *Aedes aegypti* (Diptera: Culicidae) Mosquito in El Salvador, Vector of Dengue, Yellow Fever, Chikungunya and Zika. *Parasites Vectors* **2018**, *11*, 637. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Paupy, C.; Le Goff, G.; Brengues, C.; Guerra, M.; Revollo, J.; Barja Simon, Z.; Hervé, J.P.; Fontenille, D. Genetic Structure and Phylogeography of *Aedes aegypti*, the Dengue and Yellow-Fever Mosquito Vector in Bolivia. *Infect. Genet. Evol.* **2012**, *12*, 1260–1269. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Pless, E.; Powell, J.R.; Seger, K.R.; Ellis, B.; Gloria-Soria, A. Evidence for Serial Founder Events during the Colonization of North America by the Yellow Fever Mosquito, *Aedes aegypti*. *Ecol. Evol.* **2022**, *12*, e8896. [\[CrossRef\]](#)
7. Maitra, A.; Cunha-Machado, A.S.; de Souza Leandro, A.; da Costa, F.M.; Scarpassa, V.M. Exploring Deeper Genetic Structures: *Aedes aegypti* in Brazil. *Acta Trop.* **2019**, *195*, 68–77. [\[CrossRef\]](#)
8. Rodrigues, L.F.; de Sousa, A.A.; Mendes Júnior, W.P.; Cardoso e Silva, A.C.; do Nascimento, M.H.S.; Barros, M.C.; Sampaio, I.; Fraga, E. da C. Genetic Differentiation of *Aedes aegypti* (Diptera: Culicidae) in Areas with High Rates of Infestation in Mid-North Region of Brazil. *Insects* **2023**, *14*, 530. [\[CrossRef\]](#)
9. Abuelmaali, S.A.; Jamaluddin, J.A.F.; Noaman, K.; Allam, M.; Abushama, H.M.; Elnaïem, D.E.; Ishak, I.H.; Wajidi, M.F.F.; Jaal, Z.; Kassim, N.F.A. Distribution and Genetic Diversity of *Aedes aegypti* Subspecies across the Sahelian Belt in Sudan. *Pathogens* **2021**, *10*, 78. [\[CrossRef\]](#)
10. Eskildsen, G.A.; Rovira, J.R.; Smith, O.; Miller, M.J.; Bennett, K.L.; McMillan, W.O.; Loaiza, J. 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* **2018**, *13*, e0194874. [\[CrossRef\]](#)
11. Fraga, E.C.; Oliveira, D.R.S.; Aragão, D.G.; Sampaio, H.S.; Barros, M.C. Genetic Variability and Evidence of Two Distinct Lineages of *Aedes aegypti* (Diptera, Culicidae) on São Luís Island in Maranhão, Brazil. *Open Trop. Med. J.* **2013**, *6*, 11–18. [\[CrossRef\]](#)
12. Sousa, A.A.; Fraga, E.; Sampaio, I.; Schneider, H.; Barros, M.C. Genetic Differentiation in Populations of *Aedes aegypti* (Diptera, Culicidae) Dengue Vector from the Brazilian State of Maranhão. *Rev. Bras. Entomol.* **2017**, *61*, 51–59. [\[CrossRef\]](#)
13. Avise, J.C. Phylogeography: Retrospect and Prospect. *J. Biogeogr.* **2009**, *36*, 3–15. [\[CrossRef\]](#)
14. Boore, J.L. Animal Mitochondrial Genomes. *Nucleic Acids Res.* **1999**, *27*, 1767–1780. [\[CrossRef\]](#)
15. Brown, W.M.; George, M.; Wilson, A.C. Rapid Evolution of Animal Mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 1967–1971. [\[CrossRef\]](#)
16. Moritz, C.; Dowling, T.; Brown, W. Evolution of Animal Mitochondrial DNA: Relevance for Population Biology and Systematics. *Annu. Rev. Ecol. Syst.* **1987**, *18*, 269–292. [\[CrossRef\]](#)

17. de Oliveira Aragão, A.; da Silva, F.S.; Cruz, A.C.; da Silva, S.P.; de Almeida Medeiros, D.B.; Dias, D.D.; do Nascimento, B.L.; Júnior, J.W.; de Oliveira Monteiro, H.A.; Neto, J.P. Description of Mitochondrial Genome and Phylogenetic Considerations of *Sabethes bipartites*, *Sabethes cyaneus*, *Sabethes quasicyaneus*, and *Sabethes tarsopus* (Diptera: Culicidae). *Acta Trop.* **2022**, *232*, 106493. [[CrossRef](#)] [[PubMed](#)]
18. Demari-Silva, B.; Foster, P.G.; de Oliveira, T.M.P.; Bergo, E.S.; Sanabani, S.S.; Pessôa, R.; Sallum, M.A.M. Mitochondrial Genomes and Comparative Analyses of *Culex camposi*, *Culex coronator*, *Culex usquatus* and *Culex usquatissimus* (Diptera: Culicidae), Members of the Coronator Group. *BMC Genom.* **2015**, *16*, 1. [[CrossRef](#)]
19. Hao, Y.J.; Zou, Y.L.; Ding, Y.R.; Xu, W.Y.; Yan, Z.T.; Li, X.D.; Fu, W.B.; Li, T.J.; Chen, B. Complete Mitochondrial Genomes of *Anopheles stephensi* and *An. dirus* and Comparative Evolutionary Mitochondriomics of 50 Mosquitoes. *Sci. Rep.* **2017**, *7*, 7666. [[CrossRef](#)]
20. Lemos, P.D.; Monteiro, H.A.; Castro, F.C.; Lima, C.P.; Silva, D.E.; Vasconcelos, J.M.; Oliveira, L.F.; Silva, S.P.; Cardoso, J.F.; Vianez Júnior, J.L.; et al. Characterization of Mitochondrial Genome of *Haemagogus Janthinomys* (Diptera: Culicidae). *Mitochondrial DNA Part A* **2017**, *28*, 50–51. [[CrossRef](#)]
21. Lorenz, C.; Alves, J.M.P.; Foster, P.G.; Sallum, M.A.M.; Suesdek, L. First Record of Translocation in Culicidae (Diptera) Mitogenomes: Evidence from the Tribe Sabethini. *BMC Genom.* **2019**, *20*, 721. [[CrossRef](#)] [[PubMed](#)]
22. do Nascimento, B.L.; da Silva, F.S.; Nunes-Neto, J.P.; de Almeida Medeiros, D.B.; Cruz, A.C.; da Silva, S.P.; da Silva e Silva, L.H.; de Oliveira Monteiro, H.A.; Dias, D.D.; Vieira, D.B.; et al. First Description of the Mitogenome and Phylogeny of Culicinae Species from the Amazon Region. *Genes* **2021**, *12*, 1983. [[CrossRef](#)] [[PubMed](#)]
23. da Silva, F.S.; Cruz, A.C.; de Almeida Medeiros, D.B.; da Silva, S.P.; Nunes, M.R.; Martins, L.C.; Chiang, J.O.; da Silva Lemos, P.; Cunha, G.M.; de Araujo, R.F.; et al. Mitochondrial Genome Sequencing and Phylogeny of *Haemagogus albomaculatus*, *Haemagogus leucocelaenus*, *Haemagogus spegazzinii*, and *Haemagogus tropicalis* (Diptera: Culicidae). *Sci. Rep.* **2020**, *10*, 16948. [[CrossRef](#)]
24. Silva, L.H.S.; Silva, F.S.; de Medeiros, D.B.A.; Cruz, A.C.R.; Silva, S.P.; de Aragão, A.O.; Dias, D.D.; Nascimento, B.L.S.; Rosa, J.W., Jr.; Vieira, D.B.R.; et al. Description of the Mitogenome and Phylogeny of *Aedes* spp. (Diptera: Culicidae) from the Amazon Region. *Acta Trop.* **2022**, *232*, 106500. [[CrossRef](#)]
25. Silva, F.S.; Nascimento, B.L.S.; Cruz, A.C.R.; Silva, S.P.; de Aragão, A.O.; Dias, D.D.; da Silva, L.H.S.; Reis, L.A.M.; Rosa, J.W.; Vieira, D.B.R.; et al. Sequencing and Description of the Complete Mitochondrial Genome of *Limatus durhamii* (Diptera: Culicidae). *Acta Trop.* **2023**, *239*, 106805. [[CrossRef](#)] [[PubMed](#)]
26. Rebouças, A. Água Na Região Nordeste: Desperdício e Escassez. *Estud. Avançados* **1997**, *11*, 127–154. [[CrossRef](#)]
27. Consoli, R.A.G.; Oliveira, R. *Principais Mosquitos de Importância Sanitária No Brasil*; Editora Fiocruz: Rio de Janeiro, Brazil, 1994.
28. Santos, J.M.M.; Contel, E.P.B.; Kerr, W.E. Biologia de Anofelinos Amazônicos 1—Ciclo Biológico, Postura e Estádios Larvais De. *Acta Amaz.* **1981**, *11*, 789–797. [[CrossRef](#)]
29. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An Ultra-Fast All-in-One FASTQ Preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [[CrossRef](#)]
30. Langmead, B.; Salzberg, S.L. Fast Gapped-Read Alignment with Bowtie 2. *Nat. Methods* **2012**, *9*, 357–359. [[CrossRef](#)]
31. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R. The Sequence Alignment/Map Format and SAMtools. *Bioinformatics* **2009**, *25*, 2078–2079. [[CrossRef](#)]
32. Li, D.; Liu, C.M.; Luo, R.; Sadakane, K.; Lam, T.W. MEGAHIT: An Ultra-Fast Single-Node Solution for Large and Complex Metagenomics Assembly via Succinct de Bruijn Graph. *Bioinformatics* **2015**, *31*, 1674–1676. [[CrossRef](#)] [[PubMed](#)]
33. Buchfink, B.; Xie, C.; Huson, D.H. Fast and Sensitive Protein Alignment Using DIAMOND. *Nat. Methods* **2014**, *12*, 59–60. [[CrossRef](#)] [[PubMed](#)]
34. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data. *Bioinformatics* **2012**, *28*, 1647–1649. [[CrossRef](#)] [[PubMed](#)]
35. Bernt, M.; Donath, A.; Jühling, F.; Externbrink, F.; Florentz, C.; Fritzsch, G.; Pütz, J.; Middendorf, M.; Stadler, P.F. MITOS: Improved de Novo Metazoan Mitochondrial Genome Annotation. *Mol. Phylogenet. Evol.* **2013**, *69*, 313–319. [[CrossRef](#)] [[PubMed](#)]
36. Altschul, S.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic Local Alignment Search Tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [[CrossRef](#)]
37. Stothard, P.; Wishart, D.S. Circular Genome Visualization and Exploration Using CGView. *Bioinformatics* **2005**, *21*, 537–539. [[CrossRef](#)]
38. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [[CrossRef](#)]
39. Perna, N.T.; Kocher, T.D. Patterns of Nucleotide Composition at Fourfold Degenerate Sites of Animal Mitochondrial Genomes. *J. Mol. Evol.* **1995**, *41*, 353–358. [[CrossRef](#)]
40. Yang, Z. User Guide PAML: Phylogenetic Analysis by Maximum Likelihood. *Mol. Biol. Evol.* **2007**, *4*, 1–70.
41. Katoh, K.; Misawa, K.; Kuma, K.I.; Miyata, T. MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [[CrossRef](#)]
42. Larsson, A. AliView: A Fast and Lightweight Alignment Viewer and Editor for Large Datasets. *Bioinformatics* **2014**, *30*, 3276–3278. [[CrossRef](#)] [[PubMed](#)]

43. Shen, W.; Le, S.; Li, Y.; Hu, F. SeqKit: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation. *PLoS ONE* **2016**, *11*, e0163962. [[CrossRef](#)] [[PubMed](#)]
44. Nguyen, L.T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [[CrossRef](#)] [[PubMed](#)]
45. Soares-da-Silva, J.; Ibiapina, S.S.; Bezerra, J.M.T.; Tadei, W.P.; Pinheiro, V.C.S. Variation in *Aedes aegypti* (Linnaeus) (Diptera, Culicidae) Infestation in Artificial Containers in Caxias, State of Maranhão, Brazil. *Rev. Soc. Bras. Med. Trop.* **2012**, *45*, 174–179. [[CrossRef](#)]
46. Kotsakiozi, P.; Gloria-Soria, A.; Caccone, A.; Evans, B.; Schama, R.; Martins, A.J.; Powell, J.R. Tracking the Return of *Aedes aegypti* to Brazil, the Major Vector of the Dengue, Chikungunya and Zika Viruses. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005653. [[CrossRef](#)]
47. Vargas, L.D.L.; Ferreira, S.M.B.; Souza, M.D.; da Silva, C.A.L.; Shimoya-bittencourt, W. Resistência Das Populações de *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Insecta, Diptera, Culicidae) Aos Inseticidas Utilizados Para o Controle. *Rev. Cienc. Méd. Biol.* **2022**, *21*, 98–116. [[CrossRef](#)]
48. Lima-Camara, T.N. Emerging Arboviruses and Public Health Challenges in Brazil. *Rev. Saude Publica* **2016**, *50*, 36. [[CrossRef](#)]
49. Powell, J.R. Perspective Piece Mosquito-Borne Human Viral Diseases: Why *Aedes aegypti*? *Am. J. Trop. Med. Hyg.* **2018**, *98*, 1563–1565. [[CrossRef](#)]
50. Sinclair, J.B.; Asgari, S. Ross River Virus Provokes Differentially Expressed MicroRNA and RNA Interference Responses in *Aedes aegypti* Mosquitoes. *Viruses* **2020**, *12*, 695. [[CrossRef](#)]
51. Severson, D.W.; Behura, S.K. Genome Investigations of Vector Competence in *Aedes aegypti* to Inform Novel Arbovirus Disease Control Approaches. *Insects* **2016**, *7*, 58. [[CrossRef](#)]
52. dos Paduan, K.S.; Araújo, J.P.; Ribolla, P.E.M. Genetic Variability in Geographical Populations of *Aedes aegypti* (Diptera, Culicidae) in Brazil Elucidated by Molecular Markers. *Genet. Mol. Biol.* **2006**, *29*, 391–395. [[CrossRef](#)]
53. Bracco, J.E.; Capurro, M.L.; Lourenço-de-Oliveira, R.; Sallum, M.A.M. Genetic Variability of *Aedes aegypti* in the Americas Using a Mitochondrial Gene: Evidence of Multiple Introductions. *Mem. Inst. Oswaldo Cruz* **2007**, *102*, 572–579. [[CrossRef](#)] [[PubMed](#)]
54. dos Paduan, K.S.; Ribolla, P.E.M. Mitochondrial DNA Polymorphism and Heteroplasmy in Populations of *Aedes aegypti* in Brazil. *J. Med. Entomol.* **2008**, *45*, 59–67. [[CrossRef](#)]
55. Scarpassa, V.M.; Cardoza, T.B.; Cardoso, R.P. Population Genetics and Phylogeography of *Aedes aegypti* (Diptera: Culicidae) from Brazil. *Am. J. Trop. Med. Hyg.* **2008**, *78*, 895–903. [[CrossRef](#)]
56. Monteiro, F.A.; Shama, R.; Martins, A.J.; Gloria-Soria, A.; Brown, J.E.; Powell, J.R. Genetic Diversity of Brazilian *Aedes aegypti*: Patterns Following an Eradication Program. *PLoS Negl. Trop. Dis.* **2014**, *8*, e3167. [[CrossRef](#)]
57. Matthews, B.J.; Dudchenko, O.; Kingan, S.B.; Koren, S.; Antoshechkin, I.; Crawford, J.E.; Glassford, W.J.; Herre, M.; Redmond, S.N.; Rose, N.H.; et al. Improved Reference Genome of *Aedes aegypti* Informs Arbovirus Vector Control. *Nature* **2018**, *563*, 501–507. [[CrossRef](#)]
58. Wolstenholme, D.R. Animal Mitochondrial DNA: Structure and Evolution. *Int. Rev. Cytol.* **1992**, *141*, 173–216. [[CrossRef](#)]
59. Zhang, D.X.; Hewitt, G.M. Insect Mitochondrial Control Region: A Review of Its Structure, Evolution and Usefulness in Evolutionary Studies. *Biochem. Syst. Ecol.* **1997**, *25*, 99–120. [[CrossRef](#)]
60. Makhawi, A.M.; Liu, X.B.; Yang, S.R.; Liu, Q.Y. Genetic Variations of ND5 Gene of MtDNA in Populations of Anopheles Sinensis (Diptera: Culicidae) Malaria Vector in China. *Parasites Vectors* **2013**, *6*, 290. [[CrossRef](#)]
61. Wang, G.; Li, C.; Guo, X.; Xing, D.; Dong, Y.; Wang, Z.; Zhang, Y.; Liu, M.; Zheng, Z.; Zhang, H.; et al. Identifying the Main Mosquito Species in China Based on DNA Barcoding. *PLoS ONE* **2012**, *7*, e047051. [[CrossRef](#)]
62. Xu, J.; Fonseca, D.M. One-Way Sequencing of Multiple Amplicons from Tandem Repetitive Mitochondrial DNA Control Region. *Mitochondrial DNA* **2011**, *22*, 155–158. [[CrossRef](#)] [[PubMed](#)]
63. Kambhampati, S.; Smith, P.T. PCR Primers for the Amplification of Four Insect Mitochondrial Gene Fragments. *Insect Mol. Biol.* **1995**, *4*, 233–236. [[CrossRef](#)]
64. Lorenz, C.; Alves, J.M.P.; Foster, P.G.; Suesdek, L.; Sallum, M.A.M. Phylogeny and Temporal Diversification of Mosquitoes (Diptera: Culicidae) with an Emphasis on the Neotropical Fauna. *Syst. Entomol.* **2021**, *46*, 798–811. [[CrossRef](#)]
65. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; DeWaard, J.R. Biological Identifications through DNA Barcodes. *Proc. R. Soc. B Biol. Sci.* **2003**, *270*, 313–321. [[CrossRef](#)]
66. Chan, A.; Chiang, L.P.; Hapuarachchi, H.C.; Tan, C.H.; Pang, S.C.; Lee, R.; Lee, K.S.; Ng, L.C.; Lam-Phua, S.G. DNA Barcoding: Complementing Morphological Identification of Mosquito Species in Singapore. *Parasites Vectors* **2014**, *7*, 569. [[CrossRef](#)] [[PubMed](#)]
67. Reinert, J. Comparative Anatomy of the Female Genitalia of Genera and Subgenera in Tribe Aedini (Diptera: Culicidae). Part V. Genus *Aedes* Meigen. *Contrib. Am. Entomol. Inst.* **2000**, *32*, 1–102.
68. Reinert, J. Comparative Anatomy of the Female Genitalia of Generic-Level Taxa in Tribe Aedini (Diptera: Culicidae). Part XV. Genus *Georgecraigius* Reinert, Harbach and Kitching. *Contrib. Am. Entomol. Inst.* **2008**, *35*, 1–10.
69. Reinert, J.F.; Harbach, R.E.; Kitching, I.J. Phylogeny and Classification of Tribe Aedini (Diptera: Culicidae). *Zool. J. Linn. Soc.* **2009**, *157*, 700–794. [[CrossRef](#)]
70. Wilkerson, R.C.; Linton, Y.M.; Fonseca, D.M.; Schultz, T.R.; Price, D.C.; Strickman, D.A. Making Mosquito Taxonomy Useful: A Stable Classification of Tribe Aedini That Balances Utility with Current Knowledge of Evolutionary Relationships. *PLoS ONE* **2015**, *10*, e0133602. [[CrossRef](#)]

71. Ma, X.X.; Wang, F.F.; Wu, T.T.; Li, Y.; Sun, X.J.; Wang, C.R.; Chang, Q.C. First Description of the Mitogenome and Phylogeny: *Aedes Vexans* and *Ochlerotatus Caspius* of the Tribe Aedini (Diptera: Culicidae). *Infect. Genet. Evol.* **2022**, *102*, 105311. [[CrossRef](#)]
72. Zadra, N.; Rizzoli, A.; Rota-Stabelli, O. Chronological Incongruences between Mitochondrial and Nuclear Phylogenies of *Aedes* Mosquitoes. *Life* **2021**, *11*, 181. [[CrossRef](#)] [[PubMed](#)]
73. da Brasil, M.S. Maranhão Registrou Mais de 3,1 Mil Casos Prováveis de Dengue Este Ano. Available online: <https://www.gov.br/saude/pt-br/assuntos/noticias-para-os-estados/maranhao/2023/maio/maranhao-registrou-mais-de-3-1-mil-casos-provaveis-de-dengue-este-ano> (accessed on 26 July 2023).
74. Silva, A.G.; Cunha, I.C.L.; Santos, W.S.; Luz, S.L.B.; Ribolla, P.E.M.; Abad-Franch, F. Gene Flow Networks among American *Aedes aegypti* Populations. *Evol. Appl.* **2012**, *5*, 664–676. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.