ENSINO, SAÚDE E AMBIENTE

Genética Comparada Utilizando Três Marcadores de mtDNA em Populações de *Aedes aegypti* (Linnaeus) de Municípios do Estado de Mato Grosso, Brasil

Comparative Genetics Using Three mtDNA Markers in Aedes aegypti (Linnaeus) Populations from Municipalities in the State of Mato Grosso, Brazil.

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Palavras-chave: variabilidade genética; DNA mitocondrial; ovitrampas; vetor.

Resumo: Uma estratégia metodológica para o ensino em saúde e ambiente é conhecer a biologia de um importante vetor da dengue em distintas populações. O objetivo deste estudo foi investigar a variabilidade genética de *Aedes aegypti* por meio de marcadores moleculares de DNA mitocondrial, COI, ND4 e ND5. Os mosquitos foram coletados com o auxílio de ovitrampas para captura em pontos localizados em quatro municípios do estado de Mato Grosso. Amplificamos 169 amostras com o melhor resultado de DNA utilizando *primers* para DNA mitocondrial (mtDNA): Subunidade I da Citocromo Oxidase (COI - F e R), Subunidade 4 da Nicotinamida Adenina Dinucleotídeo Desidrogenase (ND4 - F e R) e Subunidade da Nicotinamida Adenina Desidrogenase 5 (ND5 - F e R). Utilizou-se o software Geneious para construir dendrogramas a fim de diferenciar populações de cada município. A distância genética interpopulacional obtida a partir da análise de sequências mostrou diferença dentro das populações através da formação de grupos no ordenamento. Além disso, identificamos diferença nos valores de distância genética interindividual, notadamente para o gene ND5 das populações capturadas nos quatro municípios. Registramos a menor distância genética interindividual dentro de populações desses vetores no município de Chapada dos Guimarães. Fatores extrínsecos, incluindo a remoção do habitat de reprodução, podem contribuir para diminuir a variabilidade, consequentemente, o dendrograma apresentou algumas semelhanças. O conhecimento científico, o ensino em laboratório e a investigação do fluxo genético estimulam ações de prevenção de doenças transmitidas e apoiam medidas essenciais e eficazes de controle e combate ao *Ae. aegypti*.

Keywords:
genetic variability; mitochondrial DNA; ovitraps; vector.

Abstract: A methodological strategy for teaching in health and environment is to know the genetics of an important vector of dengue in different populations of mosquitoes. The aim of this study was to investigate the genetic variability of *Aedes aegypti* through molecular markers of mitochondrial DNA, COI, ND4 and ND5. Mosquitoes were collected using ovitraps to capture them at points located in four municipalities in the state of Mato Grosso. Subsequently, we amplified 169 samples with the best DNA result using primers for mitochondrial DNA (mtDNA): Cytochrome Oxidase Subunit I (COI - F and R), Nicotinamide Adenine Dinucleotide Dehydrogenase Subunit 4 (ND4 - F and R) and Nicotinamide Adenine Dehydrogenase 5 (ND5 - F and R). We used the Geneious software to build dendrograms for differentiating populations from each municipality. The interpopulational genetic distance obtained from sequence analysis showed a difference within populations through groups' formation in the ordering. Besides, we identified a difference in the interindividual genetic distance values, notably for the ND5 gene from the populations captured in the four municipalities. We recorded the smallest interindividual genetic distance within populations for populations from Chapada dos Guimarães. Extrinsic factors, including breeding habitat removal, can contribute to decreasing variability, consequently, the dendrogram showed some similarities. Scientific knowledge, laboratory teaching and genetic flow investigation stimulate actions to prevent transmitted diseases and support essential and effective measures to control and combat *Ae. aegypti*.

Introduction

Aedes (*Stegomyia*) *aegypti* mosquito Linnaeus 1762, a mosquito native to Africa (BRAAK et al. 2018), has spread throughout tropical and subtropical areas due to anthropogenic activities. Today, *Ae. aegypti* is responsible for transmitting dengue, yellow fever, Chikungunya, and Zika virus infection. Dengue is currently one of the most frequent diseases in Brazil (OLIVEIRA et al., 2020; MINISTÉRIO DA SAÚDE 2020). Scientific knowledge, laboratory teaching and genetic flow investigation stimulate actions to prevent transmitted diseases and support essential and effective measures to control and combat *Ae. aegypti* (PINHEIRO & ROCHA, 2018; FREITAS et al, 2019). Its annual variations in reporting and expansion is directly related to several factors, such as favorable environment, new serotype circulation, human population movements, and vector local infestation level. For this reason, *Ae. aegypti* adaptation to urban environments favors anthropic long-distance dispersion, supported by the eggs' resistance to desiccation and human host (SEIXAS et al. 2013).

An alternative monitoring method of *Ae. aegypti* is the detection and quantification of eggs deposited in oviposition traps installed in the home environment, which allows the identification of areas with the presence of mosquitoes and the analysis of the spatial and temporal distribution of its population (DEGENER at al. 2014). An essential tool in this vector's study is the capture employing ovitraps to control and monitor populations by obtaining a significant number of specimens. This tool provides information on the presence and population density in different environments that can be estimated using samples, based on genetic methods (FILIPOVIĆ et al. 2020). Population diagnosis of *Ae. aegypti* through ovitraps allows

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checking the presence of mosquitoes throughout the year by attracting the pregnant female to oviposition (MIYAZAKI et al. 2009). A study in China, which employed this capture method, showed that vector control is critical and sometimes the only effective way to block or decrease dengue transmission (LI et al. 2016). Infestations can increase according to higher temperatures, humidity (SANTOS et al. 2018), and disordered urbanization.

The genetic diversity of some South American populations have been assessed using a wide range of genetic markers. Mitochondrial DNA (mtDNA) genes are widely used for the identification of genetic variants, dispersal patterns, phylogeny, and population dynamic studies of *Ae. Aegypti* (PONCE et al. 2021). Besides the populational quantification of Ae. Aegypti, the literature reports the use of markers, including mitochondrial DNA (DEGENER et al. 2014, STEFFLER et al. 2016), especially the Cytochrome Oxidase I (COI) gene, widely used in culicid research, to analyze its genetic variability (NAIM et al. 2020). Molecular techniques, including the polymerase chain reaction (PCR), can contribute to the understanding of vector-human relationships and also enable the genetic study of populations (PADUAN & RIBOLLA 2009) and their diagnostic investigation in different biological samples (HIRAGI et al. 2009).

The evaluation of genetic diversity on a smaller scale, such as in cities, allows us to verify viral dispersion. *Ae. aegypti* genetic diversity analysis might be applied to know the structure of the populations to comprehend their dynamics, providing data that might lead to new control measures (LOPES et al. 2021). Several genetic mechanisms are known to generate variability within and between populations, whose differences can arise from random occurrences, including the genetic composition of specimens that disperse and create new populations (SALGUEIRO et al, 2019). In addition, there may be changes in allele frequencies resulting from occasional breeding in small (LEQUIME et al. 2016) or large populations, whose effect can be underestimated in short periods. Differences between populations under different environmental conditions for survival and reproduction (adaptive value GLORIA-SORIA et al. 2016) can accumulate and result in the development of a new species (SALGUEIRO et al. 2019).

Mitochondrial DNA (mtDNA) is an excellent target for molecular analysis due to the absence of introns, haploid inheritance, and because it contains only genes associated with mitochondrial functions. mtDNA is used in population genetic analysis and traces a species' evolutionary history (VOSSEMBERG et al, 2015). Interestingly, repair mechanisms for this molecule have not yet been described, which likely facilitates the accumulation of mutations and, consequently, the detection of intraspecific variations (VAN DE VOSSEMBERG et al,

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2015). Base substitutions, mtDNA deletions, and tRNA-encoding gene translocations cause changes in gene order between phylogenetically related organisms (SCHMIDT et al. 2018).

Given the above, studies on the genetic variability of mosquitoes transmitting prominent urban viruses are critical in warmer regions with favorable environments for *Ae. aegypti* proliferation, as in the state of Mato Grosso. Therefore, this research aimed to investigate the inter- and intra-populational genetic variability of this vector in four municipalities in Mato Grosso by using three mitochondrial DNA markers: COI, ND4, and ND5.

Methodology

Sampling and data. This study was carried out in four municipalities in the state of Mato Grosso: Cuiabá (CB), Várzea Grande (VG), Chapada dos Guimarães (CP), and Santo Antônio do Leverger (SA) (Fig. 1). We selected such regions due to their higher human population movement (CB and VG), distinct geographical position and altitude (SA, at about 100 m, and CP, at about 700 meters above sea level). The number of adults resulting from the development of eggs in the laboratory are shown in table 1.

This was a descriptive observational study with a quantitative and qualitative approach. The laboratory development was carried out with higher education students to learn about genetic techniques using biological samples of the dengue vector. Data were collected with the aid of ovitraps during December/2015 (flooding), February/2016 (flood), June/2016 (drought), and November/2016 (flood) to investigate the number (n) of copies of *Ae. aegypti* in the four municipalities (Table 2).

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Figure 1 - Graphical representation and location of the study area and collection points in the municipalities of Cuiabá (CB), Várzea Grande (VG), Chapada dos Guimarães (CP), and Santo Antônio do Leverger (SA), Mato Grosso. Legend 1. Cuiabá, triangle (CB-01, 02,03), Várzra Grande, star (VG-01, 02, 03) Chapada dos Guimarães, circle (CP-01, 02, 03), and Santo Antônio de Leverger, square (SA-01, 02, 03).

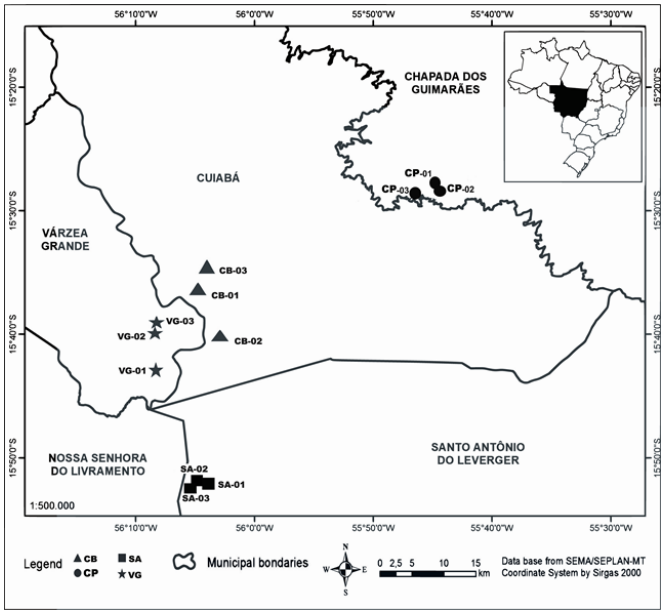

Table 1 - Number of adults resulting from the development of eggs in the laboratory. Source: IFBIOTEC Laboratory/IFMT/Cuiabá MT.

Month/Year	***Aedes aegypti samples***
December/2015	135
February/2016	355
June/2016	243
November/2016	374
Grand Total	1.107

To install the ovitraps, we selected three sampling points with a high movement of people in each municipality, as recommended by FAY & ELIASON (1966). We counted the eggs in a stereomicroscope and kept them in the laboratory for hatching and maintenance up to adulthood. We relied on relevant literature to identify adult specimens.

Then, we placed specimens in polypropylene tubes containing 100% ethanol and labeled and stored them in a freezer (-20 ºC). Individual DNA extraction from 400 *Ae. aegypti* specimens (FORATTINI, 2002) followed the manufacturer's protocol for insect DNA (Invisorb Spin Tissue Mini Kit). DNA quantification analysis was carried out after

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electrophoresis in 1% agarose gel submerged in Tris-Borate-EDTA buffer (1x) and Nano Spectrophotometer (Denovix D5). Subsequently, we amplified 169 samples with the best DNA result using primers for mitochondrial DNA (mtDNA): Cytochrome Oxidase subunit I (COI - F and R: PADUAN & RIBOLLA 2008), Nicotinamide Adenine Dinucleotide Dehydrogenase Subunit 4 (ND4 - F and R, PADUAN & RIBOLLA 2008) and Nicotinamide Adenine Dehydrogenase Subunit 5 (ND5 - F and R, BIRUNGI & MUNSTERMANN 2002). After amplification and purification, the genes underwent Sanger sequencing. We deposited the sequences on the National Center for Biotechnology Information (GenBank, www.ncbi.nlm.nih.gov) under access number (available after acceptance of the article).

We analyzed the sequencing data for COI, ND4, and ND5 genes using SeqTrace, Mega, and Geneious applications (available online and Trial version, respectively). SeqTrace was used to perform DNA strand consensus; Mega for DNA sequence, distance, and dendrogram analysis; and Geneious was used to perform sequence cutting and alignment. We checked the sequences manually using Bioedit Sequence Alignment Editor 7.0.5.2 (EDGAR 2004) Geneious® (KEARSE et al. 2012). Muscle software HALL (1999). We checked substitution saturation (transitions and transversions) of nucleotide sequence through DAMBE.

The inter and intraspecies genetic distance was determined using the classic Kimura (1980) 2-parameter (K2P) method using the nucleotide substitution model2 in MEGA v6.0 (TAMURA et al., 2013).

We tested the independent variables (variable X - sampling locations and collection months) and dependent variables (variable Y - PCR results) through:

a) analysis of variance (ANOVA) to verify the significance of data variability;

b) test for mean comparison, which consists of the smallest significant difference and amplitude of data distribution;

c) Kruskal-Wallis test (Trial version) - a multiple comparisons test -, consisting of the average between samples in the gene and base variability between populations from the municipalities. We applied this test for ANOVA treatments as the results of this analysis were significant.

We applied factorial ANOVA considering the interindividual genetic distance data based on dendrogram results per municipality. We considered gene factors (COI, ND4, and ND5) as independent variables and genetic distance as the dependent variable (factorial). The factors 'genes' and 'municipalities' were analyzed jointly using two-way ANOVA together

with nitrogenous base proportionality. We used *Ae. albopictus* as an external group and identified significant differences between each primer for the two vector species (F 1, 119 = 32,962; p = 0.000). Haplotypic diversity calculation was performed using the DNAsp 6.0 program and, haplotic maps were made by Median Joining in the NETWORK 10 program.

Results

Genetic Distance with Dendrograms. Of the 400 specimens identified as *Ae. aegypti*, 169 underwent PCR using COI, ND4, and ND5 primers. However, due to the low annealing capacity, the amplifications occurred in about 30% for COI primer, which had the lowest annealing rate (Table 2). The ND5 primer presented the best genetic variability results.

Table 2 - Number of post-sequencing samples from the four municipalities analyzed.

Municipalities Primers

Municipalities	**Primers**		
	***COI* (F/R)**	***ND4* (F/R)**	***ND5* (F/R)**
Cuiabá	7	11	10
Várzea Grande	8	11	10
Santo Antônio do Leverger	5	11	8
Chapada dos Guimarães	7	10	11
Total	**27**	**43**	**39**

Through the Geneious application, we ordered the three primers to identify the different populations in each municipality. The dendrograms ordered the specimens' distance between the municipalities (interpopulational, Fig. 2) and within each municipality (intrapopulational, Fig. 3). We also observed that the obtained sequences had interindividual differences within populations, especially when considering each municipality's sampling location. The most distant specimens indicate that there is a genetically variation, which may arise from a transitory individual into this population.

In the interpopulation analysis of the trees generated, we verified distances that only one specimen from the municipality of CB was significantly distant from the others. This finding was based on observations from the COI mitochondrial gene (Fig. 2). Similarly, one sample based on this gene in the municipality of VG. Lastly, for CP and SA municipalities,

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we did not observe any significant genetic distance among the evaluated specimens. However, intrapopulation analysis of these three genes together (Fig. 3) showed a significant distance, especially in VG municipality with COI gene.

Figure 2 - Dendrogram for the interpopulational analysis based on A) COI, B) ND4, and C) ND5 genes from Ae. aegypti populations recorded in the four municipalities. We used Ae. albopictus as an outside group for each primer. Legend 2. CB- Cuiabá; VG- Várzea Grande; CP- Chapada dos Guimarães, SA- Santo Antônio do Leverger.

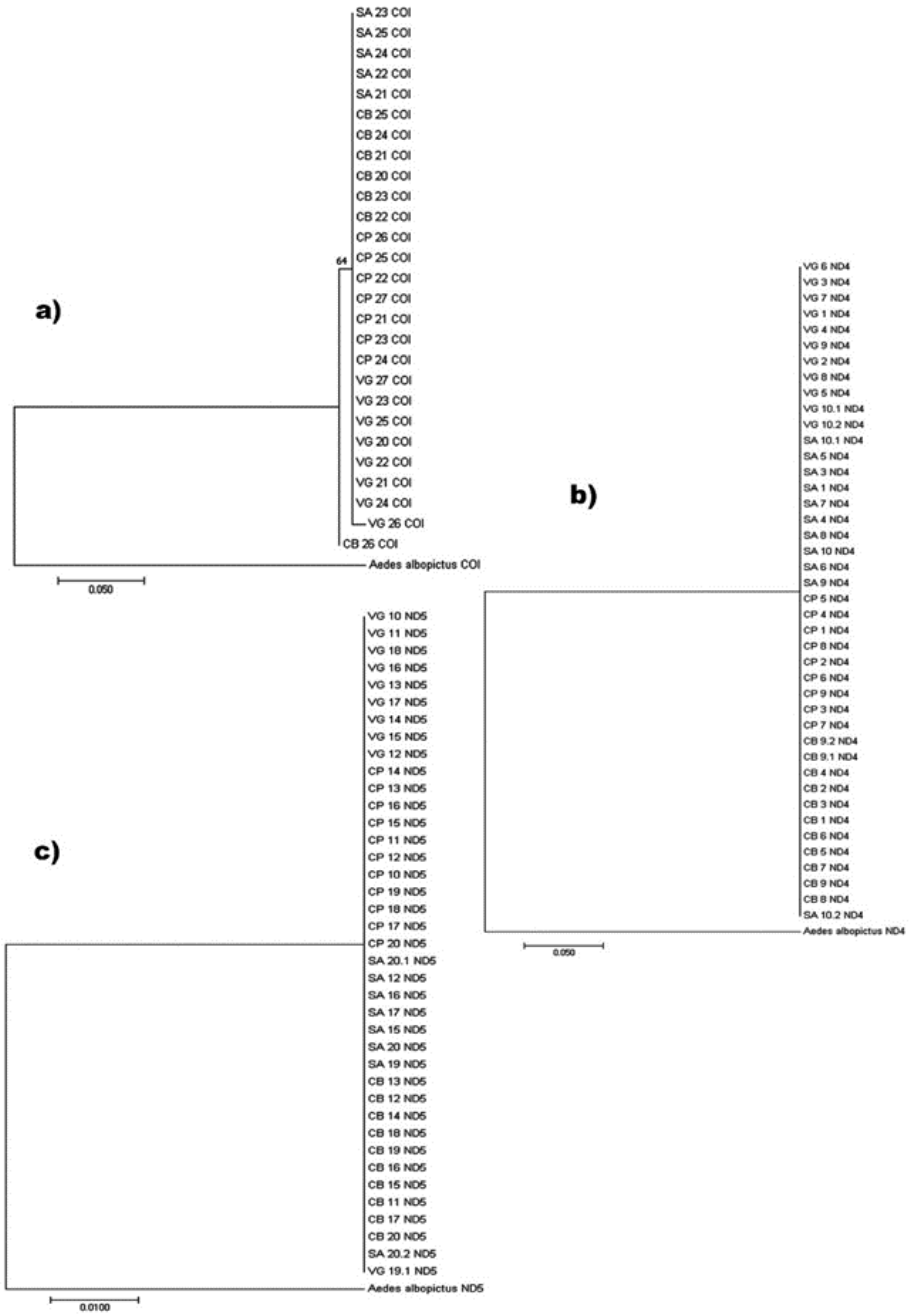

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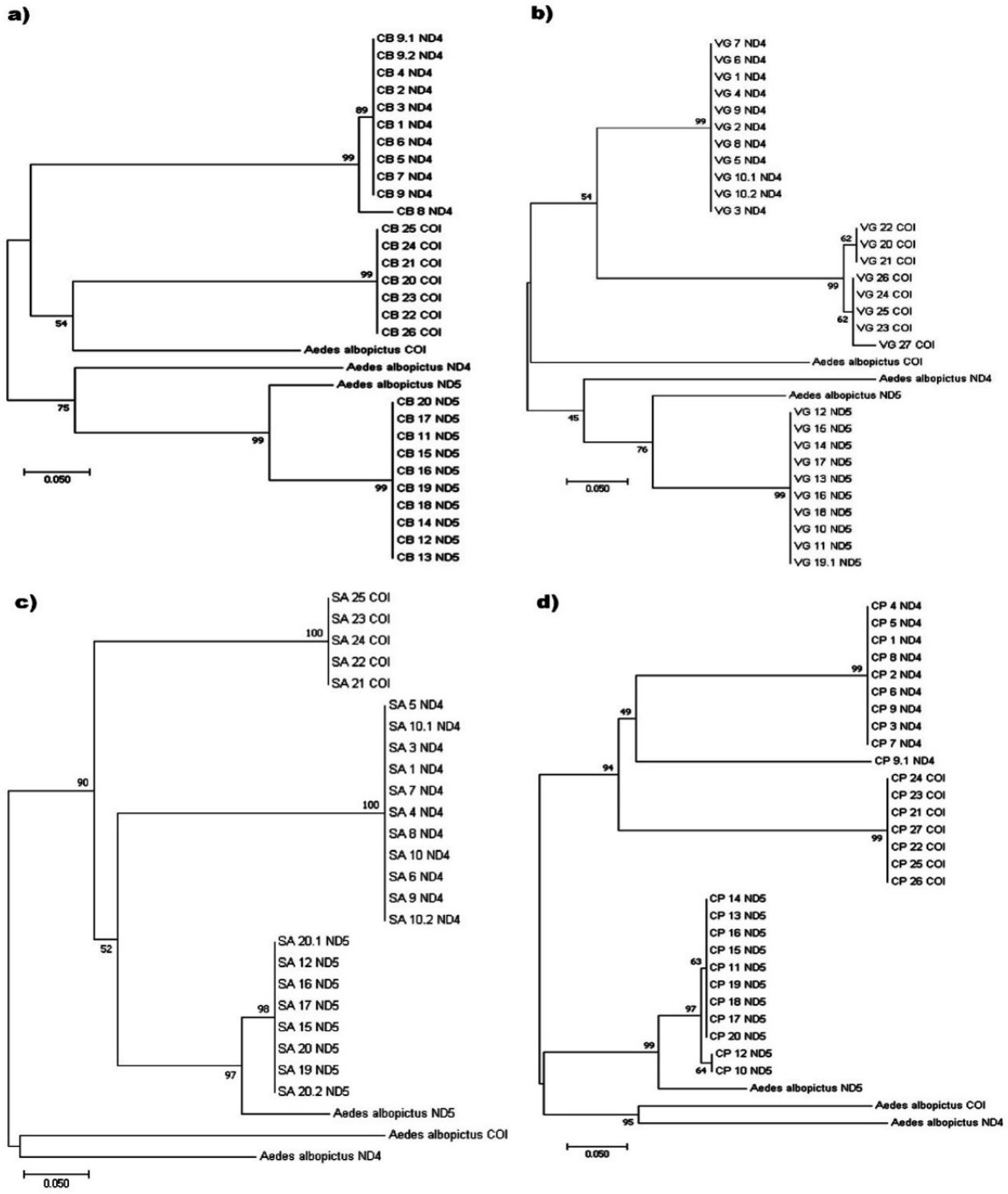
Figure 3 - Dendrogram for the intrapopulational analysis based on the results for COI, ND4, and ND5 genes from *Ae. aegypti* populations recorded in the four municipalities. We used Ae. albopictus as an outside group, using the results for each primer. Legend 3. A) CB- Cuiabá; B) VG- Várzea Grande; C) SA- Santo Antônio do Leverger; D) CP- Chapada dos Guimarães.



Genetic Distance by Analysis of Variance - ANOVA between Factors. We identified highly significant interpopulational genetic distance values between *Ae. aegypti* specimens in the different municipalities, especially for the ND5 gene (Fig. 4). The values were: CB ($F_{2,}$

$_{25}$=29003; p=0.0000), VG ($F_{2,\ 26}$=24492; p=0.0000), CP ($F_{2,\ 25}$=76,577; p=0.00000) and SA (F2, 21=50186; p=0.0000). In CP, the ND4 gene presented the lowest interpopulational genetic distance among the analyzed specimens (Fig. 5). Comparatively and significantly (F6, 97=5.4826, p=0.00006), we considered the sequence of the three primers together between the four municipalities (Fig. 6), and the municipality with the shortest distance was also CP.

We considered intrapopulational differences in the genetic distance for each gene (Fig. 5). The most significant intrapopulational genetic distance was recorded for the COI gene from specimens captured in the municipality of CB ($F_{2,\ 25}$= 34562; p=0.0000). The recording in the VG municipality was significant, with low variability for all primers ($F_{2,\ 26}$=34.069; p=0.000). In CP ($F_{2,\ 25}$=1169.2; p=0.000), the ND5 gene reached the highest genetic distance. We observed a trend of less variability in the ND5 gene in the intrapopulational genetic distance of specimens from the municipality of SA ($F_{2,\ 21}$=19.911; p=0.000).

Figure 4 - Specimens' interpopulational genetic distance mean values (standard deviation) for each gene (COI, ND4 and ND5), generated based on the dendrogram, municipalities of Cuiabá (CB), Várzea Grande (VG), Chapada dos Guimarães (CP), and Santo Antônio do Leverger/MT (SA).

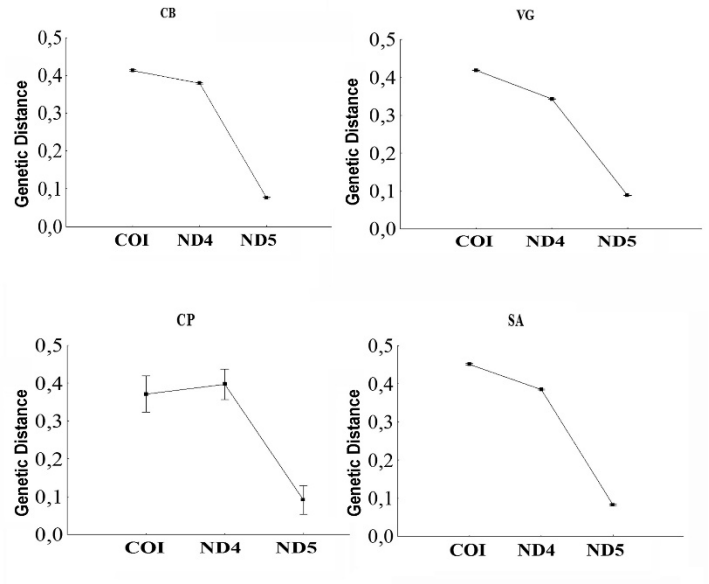


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Figure 5 - Specimens' interpopulational genetic distance mean values (standard deviation) for each gene (A) and all primers generated (B) based on the dendrogram between the 4 municipalities analyzed. Legend: Cuiabá (CB), Várzea Grande (VG), Chapada dos Guimarães (CP), and Santo Antônio do Leverger/MT (SA).

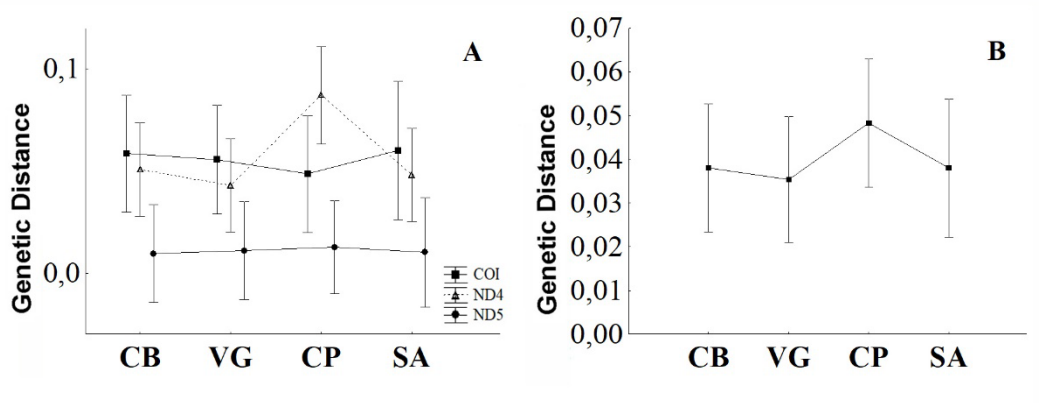
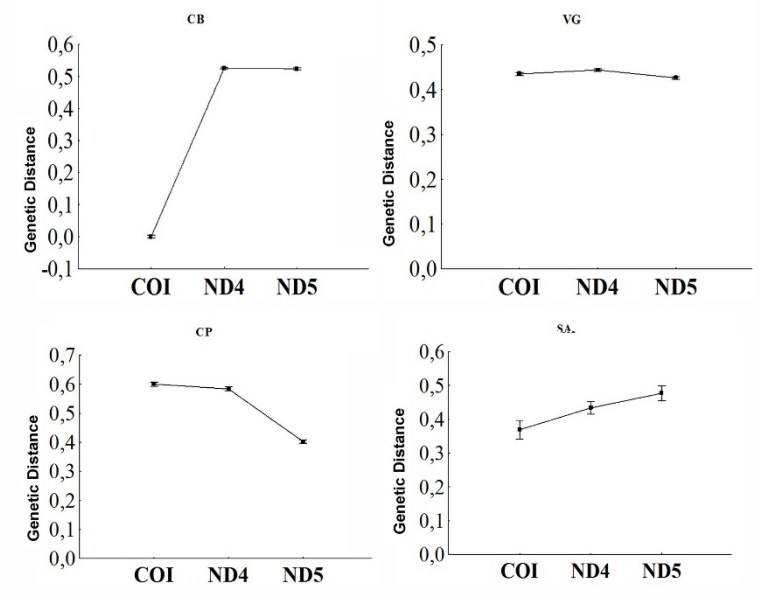


Figure 6 - Specimens' intrapopulation genetic distance mean values (standard deviation) for each gene (COI, ND4, and ND5), generated based on the dendrogram, municipalities of Cuiabá (CB), Várzea Grande (VG), Chapada dos Guimarães (CP), and Santo Antônio do Leverger (SA).



Gene polymorphisms and DNA sequencing. We sequenced the segments of the mitochondrial DNA COI, ND4, and ND5 genes using the Sanger method, whose factorial ANOVA results derived from nitrogenous bases sequencing for each gene. Based on the sequencing results of the three genes combined, the Kruskal-Wallis test demonstrated significant differences ($H_{33.52}$=69. 53; p=0.000) compared to the three genes analyzed and

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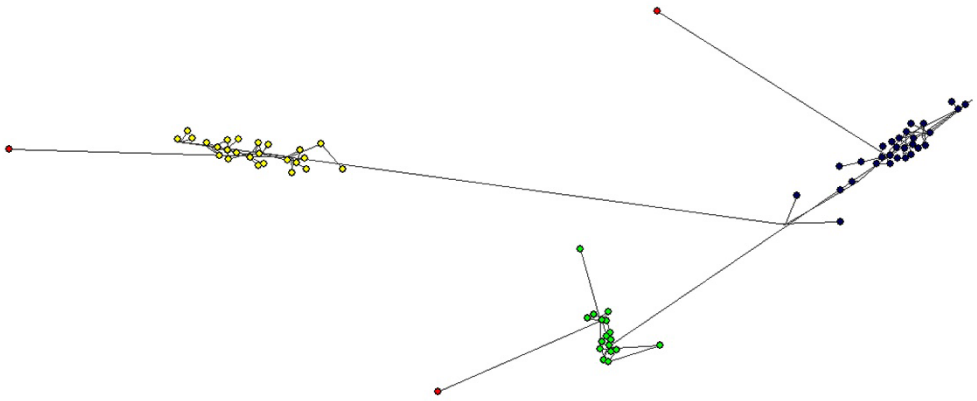
frequency values between genes (Fig. 5; $F_{8, 194}$=245.24; p=0.000). However, when we analyzed specimens from the four municipalities, the frequency values of adenine, thymine, cytosine, and guanine did not present significant differences (Fig. 6; $F_{9, 396}$=0.32682; p=0.966).

Genetic diversity values were high (h = 0.702; p = 0.015) for the four municipalities. Such analysis allowed us to identify the greatest genetic diversity, which indicated that the variation occurred within populations, with an FST value of 0.329.

Haplotipic analysis map using Median Joining. Based on genes COI, ND4, and ND5 a network haplotype of *Ae. aegypti* populations from Baixada Cuiabana has complemented this research (Fig. 7). Under this, COI was represented with a total number of mutations of S/Eta: 54, the variance of haplotype diversity: 0,01009, and nucleotide diversity, Pi: 0,02972. For ND4, the number of polymorphic (segregating) sites was S: 48, the total number of mutations Eta: 61 and, the variance of Haplotype diversity: 0,00358; the Nucleotide diversity had the Pi: 0,03916. In this same analysis for the ND5 gene, the number of polymorphic (segregating) sites was S: 19, the total number of mutations was Eta: 19 and, the variance of haplotype diversity: 0,00210 with nucleotide diversity, Pi: 0,00396.

Figure 7 - Haplotypes network map showing the threes primers, COI in yellow color, ND4 in blue and, ND5 in green color. The outgroup of Ae albopictus is shown in red with stars. Some haplotypes per each gene were significantly segregated.



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Discussion

Our study is the first to investigate the genetic variability of *Ae. aegypti* populations in municipalities of Mato Grosso. We observed a genetic variation in COI, ND4, and ND5 mtDNA sequences between specimens per analyzed location, with inter- and intrapopulational genetic distance. These genes accumulate base substitutions in the mitochondrial genome most rapidly (COSTA-DA-SILVA, et al., 2005). Populations of *Ae. aegypti* wich variability genetic influenced by seasonal phases suffer direct interference in the constitution of these organisms. This variability can be related to the resistance to population control and the spread of human diseases (LIMA et al., 2021). In this study, when populations analyzed together and by gene, the distance found unlikely represents populational fragmentation nor events that could significantly distance a representative sample of individuals.

BIRUNGI et al. (2002) investigated *Ae. albopictus* in Brazil and the United States and stated that the effect of genetic drift is more pronounced in mtDNA than in nuclear loci. The present study results showed variability in the inter- and intrapopulational genetic distance for the *Ae. aegypti* populations analyzed, notably for the ND5 gene in between municipalities' populations. *Ae. aegypti* specimen analysis confirmed that these markers are essential in population sampling.

The dispersal of mosquitoes over long distances only occurs passively (eggs and adults transported), which may explain the diversity of some haplotypes in different locations. The shortest genetic distance between genes was found for ND4 in specimens captured in CP, with an exception in one sample only. PONCE et al. 2021 compared Ecuadorian populations of Ae. aegypti from 17 sites and revealed the presence of only two haplotypes. The variations detected between ND5 gene sequences in the municipalities may indicate a genetic structure in *Ae. aegypti* resulting from several factors, including extinction and recolonization events, genetic drift, and geographical differentiation.

Interpopulational genetic distance values for the COI gene showed low variability in the municipalities. However, population genetic analyzes of *Ae. aegypti* performed by LV et al. (2020) identified a relatively high degree of polymorphism in the COI and ND4 sequences in eight populations, which were divided into eleven haplotypes. In studies using ND4 primer by SCARPASSA et al. (2008), the polymorphism values were higher for the nucleotides, the same as what we found, probably because in *Ae. aegypti* this gene is under most mutations in many samples. In some studies, distance isolation may not be significant, indicating that

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genetic distance is not always linked to geographic distance (r=-0.1216 and p=0.755, TWERDOCHLIB et al. 2012).

The genetic differentiation study of 15 populations from Maranhão1 based on the mitochondrial marker ND4, found 15 haplotypes among the polymorphic sites. It revealed that most of the variation (58.47%) was found within populations. In our study, fewer haplotypes variations occurred using ND5 and confirmed the total separation per marker applied. Of the 71 distinct haplotypes, ND4 represented 41% of the variation, COI with 35%, and ND5 with 24%. Few haplotypes were evidenced with a high mutation rate or variation about the others, within the same gene. The ND4 gene had the smallest interpopulation genetic distance among the analyzed specimens, especially in the municipality of Cuiabá.

Regarding the four municipalities analyzed in this study, the populations had specimens that were distant because of the ND5 gene, as shown by the genetic diversity values (h=0.702; p=0.015) and the FST value of 0.329, which indicated more considerable intrapopulational variation. Highly significant genetic distances can suggest speciation events. We identified a difference in the interindividual genetic distance values, notably for the ND5 gene from the populations captured in the four municipalities. Thus, our results show a robust hypothesis that new morphospecies is adapted to local environmental conditions, although morphological data is still lacking to support this assumption.

Specimens with the most considerable genetic distance may originate from distinct lineages from areas adjacent to the capture site or reflect the natural selection. They might also result from passive transport by human movement with vehicles and cargo (FRAGA et al., 2013).

The species populations from other microhabitats introduced into adjacent areas may share polymorphisms within the same and between different populations. However, the local gene flow can also be stopped due to geographical barriers; thus, no new characteristics are shared (FRAGA et al., 2013). In the present study, we found no significant barriers between populations with the highest genetic distance and intrapopulational variation.

The ordering using dendrograms with the highest genetic distance indicates the dissimilarity between some loci, as found for the ND4 gene in CP in the interpopulational analyses. Population-extrinsic factors, including breeding habitat elimination, may contribute to the low variability at some points, with some dendrogram similarity per analyzed gene. Eleven ND4 gene and 10 ND5 gene specimens formed a distinct group in the alignment of 28 individuals in the municipality of CB. Such formation confers distance and genetic variability between the specimens within the populations analyzed. Similarly, this observation was also

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present in the other investigated cities, and the ND5 gene presented variability between populations, which may be related to old and repeated introductions of the species into different habitats. SEIXAS et al (2013) support this statement in an article on the colonization and populational diversity of *Ae. aegypti* on Madeira Island, Portugal.

In the municipality of CB, we statistically recorded the highest intra-populational variability for the mitochondrial COI gene. With analyses of this gene, VAN DE VOSSEMBERG et al. (2015) identified specimens in different groups formed in dendrograms. These results are in keeping with those from this study, which can imply intraspecific variation between *Ae. aegypti* specimens. According to SEIXAS et al. (2013), different specimens analyzed based on COI exhibit greater vectorial competence than other populations with a single haplotype. The higher the genetic variability in these mosquitoes, the greater likelihood they will disperse viruses and other disease-causing parasites.

Factorial ANOVA results for the investigated genes of each *Ae. aegypti* specimen showed the differences derived from variations between populations, leading to a genetic distance within each municipality. In general, the populations' genetic profile varies by place throughout the distribution of a species. These differences can arise from random occurrences, including the genetic composition of specimens that disperse and create a new population (HIRAGI et al., 2009).

Population-intrinsic changes can suggest the influence of genetic distance, with the consequent genetic drift of rare or restricted genes to a single population or geographically close populations, as the municipalities of CB and VG. More considerable genetic distances between specimens for the ND5 gene in these municipalities can be associated with multiple introductions linked to different strains, as their habitats have high movement of people, passive dispersion patterns, and infestation control activities.

Based on nucleotide differences and sequencing, given the nitrogenous base proportionality, molecular markers did not show differences between the municipalities through the Kruskal-Wallis Test (analysis performed using the joint gene sequences). The differences were present only in the base frequency between the encoded genes. The polymorphism among *Ae. aegypti* population was greater for the ND4 gene, with a greater number of mutations and nucleotide diversity, according to the results of the haplotype network such as the template proposed by ROZAS et al. (1999). Certain specimens of *Ae. aegypti* may be more similar to each other when using COI and ND5 primers.

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SCARPASSA et al. (2008) researched in fourteen locations in Brazil, including the Cuiabá city, using mitochondrial gene Cytochrome Oxidase I (COI) to examine gene flow among 163 mosquitoes from 14 cities. Phylogenetic analysis identified two clades in genetic variability. They recorded two types of haplotypes and found a significant polymorphism among other loci. These authors' analysis revealed two strains separated by 8 fixed mutations, suggesting that *Ae. aegypti* populations likely came from eastern and western Africa, with evidence of multiple introductions.

Final Considerations

The analysis in Genetics of vectors such as *Aedes aegypti* contemplates, in a contextualized way, a formative learning that contributes to a perception of the importance of Science in vector research and a reflection on the constant variability of populations of this vector in different environments. The main form of combat is in everyone's hands.

Our findings complement the results of previous studies and have significant implications for understanding how these mosquitoes behave in different environments and under human interventions. Scientific knowledge, laboratory teaching and genetic flow investigation stimulate actions to prevent transmitted diseases and support essential and effective measures to control and combat *Ae. aegypti*.

The monitoring of *Ae. aegypti* is essential to prevent and control vector-borne infectious diseases, and governments need to design effective control measures, as mentioned by LV et al (2020). Our main limitation was that less than 20% of DNA samples were amplified using COI primers from different populations and the authors suggest that other primers need to be designed for Brazilian populations of *Ae. aegypti*.

In conclusion, this pioneering study carried out in the state of Mato Grosso shows the intrapopulation diversity of *Ae. aegypti* in each municipality investigated. Monitoring with ovitraps and genetic research of *Ae. aegypti* aimed to identify genetic variations to promote actions to prevent diseases transmitted by this species. Genetic flux and dispersion estimates can support government measures that are essential for effective vector control and combat.

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