**Advances in Bioresearch**
Adv. Biores., Vol 10 (1) January 2019: 68-79

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.10.1.6879

Advances in Bioresearch

## ORIGINAL ARTICLE

# Mitochondrial Cytochrome oxidase-I gene molecular marker used to perform the phylogenetic analysis of *Aedes aegypti* populations collected in western regions of Saudi Arabia

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**ABSTRACT**
*Mosquitoes of genus Aedes (Stegomyia) contains large number of species of which Aedesa egypti is the dramatic worldwide distribution. These Aedes species are considered of medically important vector for numerous arboviruses such as Dengue, Zika and Chikungunya viruses, which cause a huge medical problems to humans. To combat such virus diseases, the genus and species of mosquitoes must be first identify. The present investigation has aimed to perform the identification and the phylogenetic analysis of Aedesa egypti specimens collected from different western areas of Saudi Arabia using DNA sequencing assay based on cytochrome oxidase-1 (COX-1) gene. 30 mosquito samples were collected from different infected sites in Jeddah, Makkah and Taif regions. The mitochondrial COX-1 gene amplification and sequencing were carried out using the universal primers LCO1490 and HCO2198. The obtained sequences were phylogenetic analyzed and compared with available sequences in GenBank using the alignment search tool in NCBI-Nucleotide database and CLUSTAL-O (1.2.4) multiple sequence alignment. All mosquito samples were morphologically identified as Aedes (Stegomyia) aegypti. Also, all of our COX-1 gene sequences have high matching result in GenBank with KU495081.1, MF999266.1, KY022527.1, MG242463.1 and KT339669.1 of Aedes aegypti mitochondrial COX-1 gene. Aligned of all nucleotide sequences of the COX-1 gene were illustrated in a single data matrix and create two phylogeny trees. In conclusion, our results indicated that the genetic diversity and phylogenetic analysis of the collected Aedes (Stegomyia) aegypti samples may have important role in the arboviruses vector control and understanding the evolutionary processes of the Aedes cryptic species in western regions of Saudi Arabia.*
***Keywords:*** *Aedes aegypti, Morphological identification, Molecular markers, Mitochondrial COX-1 gene, Sequence alignment, Phylogenetic analysis.*

Received 04.04.2018 Revised 18.06.2018 Accepted 16.08.2018

**How to cite this article:**
M R. Al Zahrani ,E Mohammed Abdella, T Saeed Alghamdi, K M Alghamdi, J A. Mahyoub. Mitochondrial cytochrome oxidase-I gene molecular marker used to perform the phylogenetic analysis of *Aedes aegypti* populations collected in western regions of Saudi Arabia. Adv. Biores., Vol 10 [1] January 2019.68-79.

## INTRODUCTION

Several mosquito species are of medical importance because each of mosquito genera is widely distributed and becomes the major indirect cause of morbidity and mortality of human all over the world[1]; [2]. *Aedes* (Stegomyia) species especially *Aedes aegypti* and *Aedes albopictus* are the main vectors responsible for transmission of arboviruses diseases such as Dengue, Zika and Chikungunya to human and other vertebrate species [3]; [4].In addition, the world health organization (WHO, 2007) estimated that the wide spread of these mosquito species have increased public health concerns for overcome of the arbovirus related diseases and the vectors of these arboviruses [5]; [6].

In different areas of the Saudi Arabia, *Aedes aegypti* is an indigenous species and it is the main vector responsible for increase Dengue virus activity in different geographical areas [7]; [8]; [9]. Furthermore, the harmful effects of the Dengue virus transmitted by *Aedes aegypti* is increased due to there is no

vaccine for Dengue virus and lack of sustainable control measures for the vector. Kawada *et al.,* (2010) [10] exposed that, the efficient control method of this arbovirus vector may depend on the detailed knowledge of the vector population structure.

The vital step in the control of mosquito borne diseases is the identification of mosquito's genus and species. Traditionally, the identification of mosquito genus and species is mainly done on the basis of morphological characteristics. Alikhan *et al.,*[11] reported that identification of species within the subgenus Stegomyia is often based on morphological features of the adults. However, this identification method can be difficult because many of the diagnostic morphological features may be damaged during collection or storage, and also these morphological characters are not sufficient to differentiate between mosquito species, that lead to misidentification of mosquito adults collected from the fields [12]; [13].Hebert *et al.,* [14] indicated that the taxonomic methods used in morphological identification of mosquitoes vectors mainly depend on the external characters of adult females and larvae. So that these mosquito samples must be handled and stored quickly without damaging the external features. These morphological identification methods need expertise, and they are not practically possible all the time. Also, these taxonomic methods cannot identify the genetic variability and phenotypic plasticity which have an impact on genus and species identification.

Recently, DNA molecular markers have become as an efficient methods of mosquitoes genus and species identification [14]. Molecular taxonomy helps researchers to identify mosquito species, understand genetic diversity and detect phylogenetic relationships [15]. Many of the recent experiments exposed that, the mitochondrial cytochrome-c-oxidase subunit-1 (COX-1), mitochondrial dehydrogenase subunite-4 (ND-4) and the ribosomal DNA internal transcribed spacer-2 (ITS-2) genes are the most efficient genetic markers used in DNA barcoding of different mosquito species[16]; [17]; [18].

Kamgang *et al.,* [19] established that the genetic diversity and the phylogenetic relationships between different populations of Asian Triger mosquito *Aedes albopictus* have been extensively analyzed using mitochondrial DNA (mt-DNA) COX-1 gene sequences. In addition, Kumar *et al.,* [20] indicated that the molecular marker COX-1 gene is an efficient taxonomic method, which it gives an accurate solutions to the complex problems of mosquito species identification. Also, this molecular marker can detect differences through congeneric mosquito species higher than the average differences within species.

This study has aimed to improve the role of the mitochondrial cytochrome oxidase-1 (COX-1) gene sequences as a valid molecular marker for identifying and constructing the phylogenetic tree of mosquito samples of *Aedes aegypti* collected from different infected sites in Jeddah, Makkah and Taif, Saudi Arabia.

## MATERIAL AND METHODS

### Study sites and mosquitoes collection

Mosquito samples were collected from different infected spots of stagnant water occurred in three western provinces (Jeddah, Makkah and Taif) of Saudi Arabia. The sample codes and the name of the collected sites were illustrated in table (1). Mosquitoes sampling was carried out in spring of 2018 and by installing the light traps in various habitats of studying areas. Black Hole light traps (Rubicon, South Korea) were used to attract the adult mosquitoes during the entire period of the day. In addition, the powered aspirators and flash torches were used to collect the mosquitoes from their resting places.

The collected wild mosquito samples were reared in the laboratory of Experimental Station of Dengue Fever Mosquitoes, King Abdul-Aziz University in special growing cages under specific conditions (the room temperature maintained at 28 ºC and 65% room humidity). 110 mosquito adults were collected from the incubated cages manually. Only 30 mosquito samples not feed on blood were separated in 1ml Eppendorf tubes and stored at -80 ºC until use for total DNA extraction and phylogenetic analysis(Table 1).

### Morphological identification

The wild live adult mosquitoes collected from the traps in different areas were brought to the laboratory for morphological identification. After keeping these mosquito samples for 30 minutes at -24 ºC for immobilization. Mosquito identification was done on the basis of adult female morphological features using the different standard taxonomic keys [21]; [22]; [23].

### Total DNA extraction

30 adult mosquito samples were used in this experiment for total DNA extraction. The genomic DNA extraction and purification were performed following the procedures of Kress and Erickson (2012) [24] and also our previous optimized protocol for DNA extraction from individual whole mosquito [25]. Each adult mosquito was homogenized in 50 µl of insect lysis buffer (16.5 gm of GuSCN, 12 ml of 0.5M EDTA pH 8.0, 6 ml of 1M Tris-HCl pH 8.0, 1 ml Triton X-100, 10 ml Tween-20 and complete the volume to 200 ml by ddH2O). All mosquito homogenates were incubated in water bath for 60 minutes at 58 ºC with

quick addition of 5 µl of proteinase-K for each homogenate during incubation. Then these tubes incubated in ice for 30 minutes before centrifugation at 10000 rpm for 10 minutes. The clear supernatant in each tube was transferred into fresh 1.5 ml Eppendorf tube. For precipitate and purify the DNA from this supernatant was performed following the modified protocol of Abdella *et al.,* [25] using 100% ethanol and Silica Gel spin column respectively. The purified DNA samples stuck on the silica gel of the column were washed twice by adding 500 µl of 70% ethanol to each spin column and then centrifuge these columns for two minutes at 10000 rpm after each wash. Finally, transfer these columns to new 1.5 ml Eppendorf tubes and solubilized DNA by adding 50 µl ddH2O to the spin column. To collect the purified DNA, centrifuge these tubes for one minute at 9000 rpm. The solubilized DNA samples were preserved at -24 °C until molecular analysis.

The mitochondrial cytochrome-c oxidase-I (COX-1) gene was used to examine sequences polymorphism in between the collected mosquito samples. 30 total DNA extracts were used as templates to amplify $\simeq$ 710 bp fragments, including primers, specific to COX-1 gene of mt-DNA. This COX-I gene fragment was amplified by using the DNA primers pairs, LCOI490(forward primer): (5´-GGTCAACAAATCATAAAGATATTGG-3')and HCO2198 (reverse primer): (5´-TAAACTTCAGGGTGACCAAAAAATCA-3'), that are derived from Guo *et al.,* (2018) [26].

**Table 1: *Aedes* mosquito samples collected from different western regions in Saudi Arabia**

| Sample Number | Sample Code | City of sample collection | Site of sample collection | Season of sample collection |
|---|---|---|---|---|
| **1** | A1_LCO1490 | Makkah | Aleazizi | spring season |
| **2** | A2_LCO1490 | Makkah | Aleazizi | spring season |
| **3** | A3_LCO1490 | Makkah | Almusfala | spring season |
| **4** | A4_LCO1490 | Makkah | Almusfala | spring season |
| **5** | A5_LCO1490 | Makkah | Alshawqia | spring season |
| **6** | A6_LCO1490 | Makkah | Alshawqia | spring season |
| **7** | A7_LCO1490 | Makkah | Easfan | spring season |
| **8** | A8_LCO1490 | Makkah | Easfan | spring season |
| **9** | A9_LCO1490 | Makkah | Almaeabideh | spring season |
| **10** | A10_LCO1490 | Makkah | Almaeabideh | spring season |
| **11** | A11_LCO1490 | Taif | Alhawayuh | spring season |
| **12** | A12_LCO1490 | Taif | Alhawayuh | spring season |
| **13** | B1_LCO1490 | Taif | Alqimaria | spring season |
| **14** | B2_LCO1490 | Taif | Alwisam | spring season |
| **15** | B3_LCO1490 | Taif | Almuntazuh | spring season |
| **16** | B4_LCO1490 | Taif | Alwasha | spring season |
| **18** | B5_LCO1490 | Taif | Albalad | spring season |
| **19** | B6_LCO1490 | Taif | Albalad | spring season |
| **20** | B7_LCO1490 | Taif | Alrodaf | spring season |
| **22** | B9_LCO1490 | Jeddah | Aljanoob | spring season |
| **23** | B10_LCO1490 | Jeddah | khuzam | spring season |
| **24** | B11_LCO1490 | Jeddah | Al metar | spring season |
| **25** | B12_LCO1490 | Jeddah | Bryman | spring season |
| **26** | C1_LCO1490 | Jeddah | Al aziziyah | spring season |
| **27** | C2_LCO1490 | Jeddah | Alsharafiyah | spring season |
| **28** | C3_LCO1490 | Jeddah | Albalad | spring season |
| **29** | C4_LCO1490 | Jeddah | Aljamaeiah | spring season |
| **30** | C5_LCO1490 | Jeddah | Umasiam | spring season |

**Amplification and sequencing of mitochondrial COX-1 gene**

PCR amplification reaction was performed in 25 µl PCR reaction mixture containing 10-30 ng of template DNA, 0.1 mM dNTPs, 1.24 U *Taq* DNA polymerase, 1.5 mM MgCl2, 1X reaction buffer, 5 pmol of each forward and reverse primers, and sufficient nuclease-free water to make 25 µl (Invitrogen, Cergy Pontoise, France). Amplification were occurred using the following cycle conditions: an initial denaturation at 95 ºC for 1 minute followed by five cycles of 94 °C for 40 sec. (denaturation), 45 ºC for 40 sec. (annealing), and 72 ºC for 1 min. (extension); then 32 cycles of denaturing at 94 °C for 30 sec., annealing at 53 ºC for 40 sec., extension at 72 °C for 1 min.; and followed by a final extension step by

holding the reaction at 72 ºC for 10 minutes. The PCR amplification products were confirmed by run on 1.5% agarose gel to integrity, stained with ethidium bromide and visualized in a gel imaging system.
PCR amplification products showing positive clear bands in agarose gel were purified using a gel purification kit (Hilden, Germany) and given for commercial sequencing (Macrogen, Beotkkot-ro, Geumcheon-gu, Seoul, Korea) using the PCR primers in both directions.

**Phylogenetic analysis**

The obtained sequences of COX-1 gene were used to study the evolutionary relationships between the mosquito individuals sampled in the western areas of Saudi Arabia. Also, these sequences were compared with *Aedes aegypti* samples selected from the GenBank entries using BLAST software program for online phylogenetic analysis, embedded in PubMed from National Center for Biotechnology Information (blast.ncbi.nlm.nih.gov/Blast. cgi).
Mitochondrial COX-1 gene sequences of the collected 28 wild *Aedes aegypti*samples and the selected five GenBank sequences of *Aedes aegypti* (Table 2) were aligned and the phylogenetic trees were constructed by using Beast-2 software program (http://www.beast2.org.com) and Fig-tree v1.4.3 (Institute of Evolutionary Biology, University of Edinburgh). Also seven DNA sequences of our samples were performed to check for sequence homology with three mitochondrial COX-1 gene sequences (MG242463.1, MF999266.1 and KT339669.1) available in GenBank using CLUSTAL-O (1.2.4) multiple sequence alignment (European Bioinformatics Institute) in the online website (https://www.ebi.ac.uk/Tools/msa/clustalo/).

## RESULTS AND DISCUSSION

The mosquito species of genus *Aedes* (subfamily: Culicinae) are an important vector for human public health. Also they are highly invasive and the most widely distributed mosquito species around the world. Therefore, the taxonomic status and the distribution patterns of these mosquito species are the first step in the control of such mosquitoes and their transmitted diseases. In the present investigation both morphological and molecular assays were used to identification and phylogenetic analysis of the mosquitoes belonging to *Aedes* species, which were collected from the western areas in Saudi Arabia.

**Morphological identification**

Adult female mosquitoes collected from different sites in the field were all morphologically identified as members of *Aedes aegypti* subgroup using various morphological features (Table 1). As shown in figure (1), adult females of *Aedes aegypti* are smallish dark mosquito with conspicuous white markings and bands at the bases of the tarsal leg segments. The proboscis is all black although the palps are white tipped. The scutum on the dorsal surface of the thorax has a dorsal pattern of white scales in the form of violin or lyre with curved lateral and 2 central stripes contrasting with the general covering of narrow dark black or brown scales. Wings of adult females are dark scaled and also their femurs of the hind legs have pale scaled for basal three quarters with dark scales dorsally on apical two-thirds and ventrally on apical third. In addition, tibia is dark but tarsi with pale basal bands. The abdominal tergites have median and lateral white scale patches or bands (possibly some white scales on apical margins), also sternites predominantly pale scaled with subapical bands on distal segments. Since such morphological identification is not sufficient and not accurate to separate the adult females within the *Aedes* species subgroups.
The morphological identification of *Aedes* species distributed in Saudi Arabia had studied by several researchers. Godsy *et al.,* [27] and Al Kherji [28] were identified *Aedes unilineatus* in Makkah province and *Aedes caspius* in Riyadh province, respectively. While Al Ahmad *et al.,* [7] reported *Aedes aegypti* in Jeddah province. Indeed, previous study in the western regions of Saudi Arabia showed that four different species of *Aedes* were morphologically identified [11]. They investigated that, *Aedes* (Stegomyia) *aegypti* was the most abundant *Aedes* species recorded in almost all locations of Jeddah (about 57%). These results confirm our morphological identification results of all the mosquito samples that were identified as *Aedes* (Stegomyia) *aegypti*.
Recently, morphological characteristics have not been used alone as major method for mosquito genus and species identification. Furthermore, these morphological methods are not able to distinguish mosquitoes among cryptic species. Lukhtanove *et al.,* [29] and Muangami *et al.,* [30] reported that, mosquito cryptic species often are so similar, so that they cannot be distinguished via traditional methods of mosquito species identification using the morphological features. However, with the advances in molecular diagnosis technology, the molecular assays especially PCR-based techniques and DNA sequencing technology become a viable tool for taxonomic differentiation and declare the genetic diversity and phylogeny between the cryptic species [31]; [32].

**COX-1 gene amplification and sequencing**

In the present experiment, 30 extracted DNA samples were used for amplification of the mitochondrial cytochrome-c oxidase-1 (COX-1) gene by polymerase chain reaction (PCR). These COX-1 amplified gene was used in the molecular identification of mosquito species and also in phylogenetic analysis. As shown in figure (2), only 28 mosquito specimens had good amplified $\simeq$ 710 bp bands of COX-1 target gene which it is a specific molecular marker to *Aedes aegypti*. These 710 bp DNA fragments were purified from agarose gel and re-amplified before sequencing. The obtained sequences were AT-rich with an average of 60% AT content for all codes.

Molecular markers constitute one of the useful tool for entomological studies because these specific molecular markers must demonstrate the genetic diversity within the closely related mosquito species [33]. Several genetic markers have been studied in the previous investigations, such as internal transcribed spacer (ITS), mitochondrial cytochrome-c oxidase subunite-1 (COX-1) gene and mitochondrial dehydrogenase subunite-4 (ND4) gene are largely used in molecular taxonomy for detection the genetic structures of the mosquito vector species populations, and also for identification of phylogenetic relationships among or within cryptic species of Culicidae [34]; [35]; [36].

However, recent molecular taxonomic experiments investigated that the mitochondrial genes were considered good molecular markers for mosquito species identification and phylogenetic analysis due to lack of introns, limited chance to recombination and haploid nature of inheritance [31]. Previous study occurred in Central Africa based on polymorphisms to COX-1 gene sequences indicated that, the mosquitoes populations in Cameroon were identified as *Aedes albopictus* and they were genetically related to tropical mosquito populations rather than temperate or subtropical outgroups [19]. Also Abdella *et al.,* [25] recently indicated that sequence alignment and phylogenetic analysis of mitochondrial COX-1 gene sequences can be used as a useful molecular marker for identification of *Culex* species complexes.

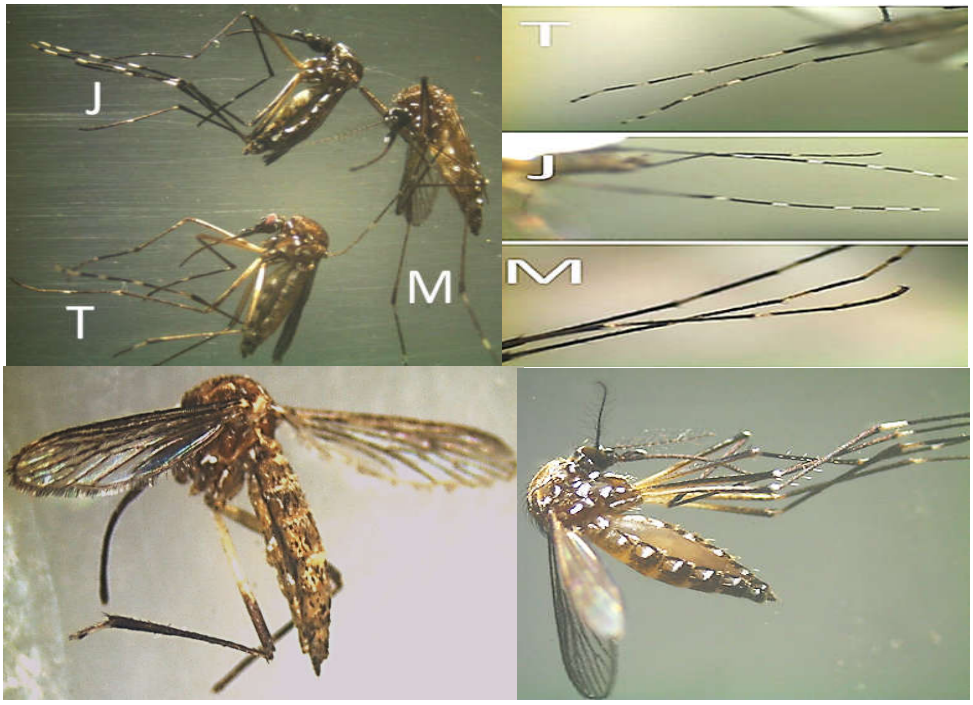


**Figure 1:**Light microscope images of wild adult female of *Aedes aegypti* mosquitoes collected from the traps indifferent areas of Jeddah (J), Makkah (M) and Taif (T) expose various morphological features including: the bands at the bases of the tarsal leg segments,the scutumon thedorsal surface of the thorax,dark scaled wings, median and lateral white scale patches or bands of the abdominal tergites and femurs of the hind legs have pale scaled for basal three quarters with dark scales dorsally on apical two-thirds and ventrally on apical third.

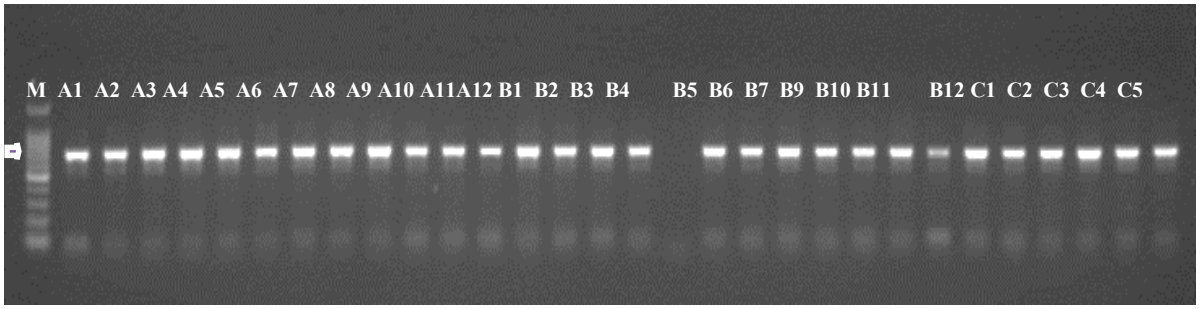


**Figure 2:** Agarose gel picture expose the amplification of ~710 bp COX-I gene in 28 mosquitoes specimens isolated from different western regions in Saudi Arabia. The arrow indicate 710 bp fragments and M is 100bp DNA ladder.

**DNA sequences blast and phylogenetic analysis**

The twenty eight obtained COX-1 gene sequences of the present samples were compared with the available previous sequences in Basic Local Alignment Search Tool (BLAST) program (blast.ncbi.nlm.nih.gov/Blast.cgi).The blast analysis results were confirmed the morphological identification, all 28 sequences show high similarity with the mitochondrial COX-1 gene sequences of *Aedes aegypti* samples in NCBI database, with an identity of 97% to 100% (Table 2 and 3).

Data illustrated in table (3) exposed that, all of our COX-1 gene sequences have high matching result in gene-bank with KU495081.1, MF999266.1, KY022527.1, MG242463.1 and KT339669.1 of *Aedes aegypti* mitochondrial COX-1 gene for cytochrome-c oxidase subunit-1. However, specimens A6_LCO1490 and A7_LCO1490 have no similarity with KT339669.1 and MG242463.1 respectively. In addition, specimens B9_LCO1490, B11_LCO1490, B12_LCO1490 and C1_LCO1490 have no significant matching with gene-bank sample MF999266.1.

Aligned nucleotide sequences of the COX-1 gene were illustrated in a single data matrix and create a phylogeny tree using Beast-2 and Figure tree v1.4.3 programs. Two phylogenetic trees were constructed for *Aedes aegypti* samples collected from Jeddah, Makkah and Taif, Saudi Arabia.

First phylogenetic tree exposed the homology relationships within the 28 examined specimens (Figure 3). These examined samples were divided into two main clusters with sister branches. *Aedes aegypti* specimens A10, B10, A12, A3, B11, B9, B12, B7, B3, A6 and B4 shared high similarity and branched as out group in the tree. While A11, A8, C3, C1, C5, A2, A4, A1, B2, B6, A5, B5, B1, C4, A7, A9 and C2clustered in one group, indicated that they have high sequence diversity from the rest of the specimens.

However, the second phylogenetic tree was constructed based on the results of the phylogenetic analysis of our 28 COX-1 gene sequences checked with GenBank samples KU495081.1, MF999266.1, KY022527.1, MG242463.1 and KT339669.1 of *Aedes aegypti* mitochondrial COX-1 gene for cytochrome-c oxidase subunit-1 (Figure 4). This phylogenetic analysis reveals that, samples A12, B3, B11 and C3 are more close to KT339669.1 (located in south India), A8 and B7 more close to MF999266.1 (India), A3, B10, A7, A6 and C2 close to KU495081.1 (located in southeastern Australia) and KY022527.1 (Germany) and also samples A1, B1 and B2 were more close to MG242463.1 (located in New Haven, USA) (Table 3).

Further, mitochondrial COX-I sequences of *Aedes aegypti* samples A6, A7, A10, B9, B11, B12 and C1 were aligned with the sequences ofMG242463.1, MF999266.1 and KT339669.1 by nucleotides using multiple sequence alignment on CLUSTAL-O (1.2.4) (European Bioinformatics Institute) in the online website (https://www.ebi.ac.uk/Tools/msa/clustalo/). As shown in figure 5, alignment of our sequences with *Aedes aegypti* sequences in GenBank(MG242463.1, MF999266.1 and KT339669.1) showed some similarities, variations and mutations which may give variability with specimens A6, A10 and B11. However, sequences of A7, B9, B12 and C1 specimens seems closer to MG242463.1, MF999266.1 and KT339669.1.Phylogenetic analysis reveals that all the sequences were branchedas outgroup in the tree as shown in Figure 6. In addition each of A6 and A10 clustered in separate clusters; indicates that they have sequence diversity from the rest of the specimens andMG242463.1, MF999266.1 and KT339669.1.

Unexpectedly, the present study shows that most of *Aedes* (Stegomyia) *aegypti* samples collected from the western regions of Saudi Arabia have high genetic similarity with Indian and Australian ancestral clade. These results suggest that the mosquito vector of arboviruses might have been introduced into Saudi Arabia by pilgrims and continued to circulate in western regions of Saudi Arabia. Also our results are confirmed by the results of Al Ali *et al.,* [9] and Weeraratne *et al.,* [37].

**Table 2: Five *Aedes aegypti* samples selected from the GenBank entries using BLAST software program for online phylogenetic analysis.**

| Genebank Code | Gene Published date | Organism and Gene Definition | Gene Length (bp) | Country | References |
|---|---|---|---|---|---|
| **KU495081.1** | 25-MAY. 2016 | *Aedes aegypti* voucher MA11647/2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | 687 | South-eastern Australia | Batovska, J., Blacket, M.J., Brown, K. and Lynch, S.E. (2016): Molecular identification of mosquitoes (Diptera: Culicidae) in southeastern Australia. Ecol. Evol., 6 (9): 3001-3011. |
| **MF999266.1** | 03-DEC. 2017 | *Aedes aegypti* cyto-chrome oxidase subunit I (COI) gene, partial cds; mitochondrial | 709 | India | Balaji, S. and Prabagaran, S.R. (Direct Submission): Submitted (26-SEP-2017) Department of Biotechnology,Bharathiar University, Marudhamalai Main Road, Coimbatore, Tamil Nadu 641046, India. |
| **KY022527.1** | 24-FEB. 2017 | *Aedes aegypti* voucher MA11647/2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | 709 | Germany | Kampen, H., Jansen, S., Schmidt-Chanasit, J. and Walther, D. (2016): Indoor development of *Aedes aegypti* in Germany. Euro. Surveill., 21 (47). |
| **MG242463.1** | 08-JAN. 2018 | *Aedes aegypti* cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | 676 | New Haven, USA | Soghigian, J., Andreadis, T.G. and Livdahl, T.P., (2017): From ground pools to treeholes: convergent evolution of habitat and phenotype in *Aedes* mosquitoes. BMC Evol. Biol. 17 (1): 262. |
| **KT339669.1** | 03-DEC. 2015 | *Aedes aegypti* voucher BU-Zoo-Ae.a-17 cyto-chrome oxidase subunit I (COI) gene, partial cds; mitochondrial | 648 | South India | Vadivalagan, C., Karthika, P., Murugan, K., *et al.,* (2015): Genetic deviation in geographically close populations of the dengue vector *Aedes aegypti* (Diptera: Culicidae): influence of environmental barriers in South India. Parasitol. Res. |

## CONCLUSION

Results of the present investigation confirmed that mitochondrial molecular markers can be successfully used for identification and phylogenetic analysis of mosquito cryptic species. Our results indicated that the genetic diversity and phylogenetic analysis of *Aedes* (Stegomyia) *aegypti* samples in western regions of Saudi Arabia appeared to be separated by a single mutation step at the mitochondrial DNA COX-1 gene. These results may have important role in the arboviruses vector control and understanding the evolutionary processes of the *Aedes* species. Low genetic polymorphisms between our *Aedes* (Stegomyia) *aegypti* samples indicated the recent introduction of this mosquito species into the western areas of Saudi Arabia. The wide spread of this invading *Aedes* species could change the epidemiology of the arbovirus diseases in Saudi Arabia. So that the mosquito vector control programs must be planned continually because the control measures of these programs may not be equally effective for genetically different mosquito populations.

**ACKNOWLEDGEMENTS**

Authors are thankful to the team of the Research Unit of Dengue Fever and Vector Control, at King Abdulaziz University(Jeddah, Saudi Arabia), for their support and providing facilities to complete this experiment. Also, we wish to extend our gratitude to Dr. Ahmed Atif and Mr. Mohammed Almatary from Genome lab at King Abdulaziz University for their training support for this research. In addition, especial grateful to Preventive Medicine Center in Taif Province for their help and cooperation.

**COMPETING INTERESTS**

The authors have declared that no competing interest exists.

**Table 3: COX-1 gene sequences blast results of 28 *Aedes aegypti* collected samples with five *Aedes aegypti* samples selected from the GenBank entries using NCBI blast-n database for online phylogenetic analysis.**

| *S.Code | KU495081.1 | | | MF999266.1 | | | KY022527.1 | | | MG242463.1 | | | KT339669.1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total Score | *Q.C. % | *Simi % | Total Score | *Q.C. % | *Simi % | Total Score | *Q.C. % | *Simi % | Total Score | *Q.C. % | *Simi % | Total Score | *Q.C. % | *Simi % |
| **A1** | 1110 | 89 | 98 | 1106 | 91 | 97 | 1106 | 91 | 97 | 1067 | 90 | 95 | 1067 | 84 | 99 |
| **A2** | 1148 | 90 | 99 | 1144 | 90 | 99 | 1144 | 90 | 99 | 1099 | 90 | 97 | 1104 | 87 | 99 |
| **A3** | 1157 | 89 | 99 | 1155 | 90 | 99 | 1155 | 90 | 99 | 1108 | 89 | 97 | 1113 | 86 | 99 |
| **A4** | 1164 | 89 | 99 | 1162 | 91 | 99 | 1162 | 91 | 99 | 1115 | 89 | 97 | 1121 | 86 | 99 |
| **A5** | 1155 | 92 | 99 | 1160 | 94 | 99 | 1160 | 94 | 99 | 1112 | 93 | 97 | 1119 | 89 | 99 |
| **A6** | 1097 | 79 | 98 | 1092 | 79 | 97 | 1092 | 79 | 97 | 1103 | 80 | 97 | -- | -- | -- |
| **A7** | 1150 | 89 | 99 | 1153 | 89 | 99 | 1153 | 89 | 99 | -- | -- | -- | 1117 | 86 | 99 |
| **A8** | 1144 | 88 | 99 | 1144 | 90 | 98 | 1144 | 90 | 98 | 1095 | 88 | 97 | 1099 | 85 | 99 |
| **A9** | 1124 | 90 | 99 | 1133 | 92 | 99 | 1133 | 92 | 99 | 1083 | 89 | 97 | 1092 | 86 | 99 |
| **A10** | 1142 | 91 | 99 | 1151 | 92 | 99 | 1151 | 92 | 99 | 1095 | 87 | 98 | 1103 | 84 | 100 |
| **A11** | 1139 | 89 | 99 | 1141 | 91 | 98 | 1141 | 91 | 98 | 1092 | 86 | 97 | 1095 | 84 | 99 |
| **A12** | 1141 | 86 | 99 | 1146 | 88 | 99 | 1146 | 88 | 99 | 1097 | 85 | 97 | 1104 | 83 | 99 |
| **B1** | 1150 | 80 | 99 | 1153 | 80 | 99 | 1153 | 80 | 99 | 1108 | 81 | 97 | 1117 | 77 | 99 |
| **B2** | 1180 | 91 | 99 | 1186 | 92 | 99 | 1186 | 92 | 99 | 1131 | 91 | 97 | 1135 | 88 | 99 |
| **B3** | 1169 | 90 | 99 | 1177 | 90 | 99 | 1177 | 90 | 99 | 1128 | 90 | 97 | 1131 | 86 | 99 |
| **B4** | 1135 | 85 | 99 | 1141 | 87 | 99 | 1141 | 87 | 99 | 1092 | 85 | 97 | 1101 | 82 | 99 |
| **B5** | 1157 | 88 | 99 | 1162 | 88 | 99 | 1162 | 88 | 99 | 1113 | 88 | 97 | 1122 | 85 | 99 |
| **B6** | 1133 | 88 | 99 | 1144 | 90 | 99 | 1144 | 90 | 99 | 1090 | 89 | 96 | 1099 | 85 | 99 |
| **B7** | 1169 | 88 | 99 | 1175 | 88 | 100 | 1175 | 88 | 100 | 1126 | 88 | 98 | 1133 | 85 | 99 |
| **B9** | 1131 | 88 | 99 | -- | -- | -- | 1137 | 88 | 100 | 1097 | 90 | 97 | 1104 | 86 | 100 |
| **B10** | 1177 | 89 | 99 | 1189 | 90 | 99 | 1189 | 90 | 99 | 1130 | 89 | 97 | 1135 | 85 | 99 |
| **B11** | 1079 | 85 | 99 | -- | -- | -- | 1088 | 86 | 99 | 1034 | 84 | 97 | 1041 | 81 | 100 |
| **B12** | 1159 | 87 | 99 | -- | -- | -- | 1169 | 88 | 99 | 1112 | 87 | 97 | 1112 | 84 | 99 |
| **C1** | 1131 | 90 | 99 | -- | -- | -- | 1137 | 90 | 99 | 1090 | 91 | 97 | 1101 | 87 | 99 |
| **C2** | 1144 | 89 | 99 | 1151 | 90 | 99 | 1151 | 90 | 99 | 1097 | 88 | 97 | 1106 | 85 | 99 |
| **C3** | 1168 | 91 | 99 | 1177 | 91 | 99 | 1177 | 91 | 99 | 1121 | 90 | 97 | 1130 | 87 | 99 |
| **C4** | 1162 | 89 | 99 | 1169 | 91 | 99 | 1169 | 91 | 99 | 1117 | 89 | 97 | 1126 | 86 | 99 |
| **C5** | 1153 | 88 | 99 | 1159 | 90 | 99 | 1159 | 90 | 99 | 1110 | 89 | 97 | 1117 | 85 | 99 |

*** S.: Sample, Q. C.: Query Cover, Simi: Similarity**

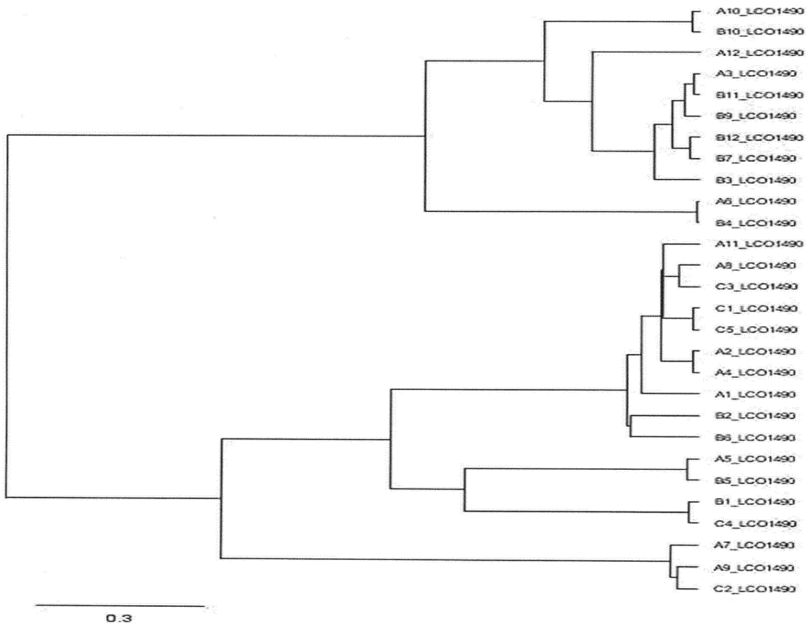


**Figure 3:** Phylogenetic analysis of 28 mitochondrial COX-I gene sequences of 29 mosquito specimens isolated from different western regions in Saudi Arabia. For phylogenetic analysis. Beast2 program (https://www.beast2.org.com) and Fig tree V1.4.3 program were used.

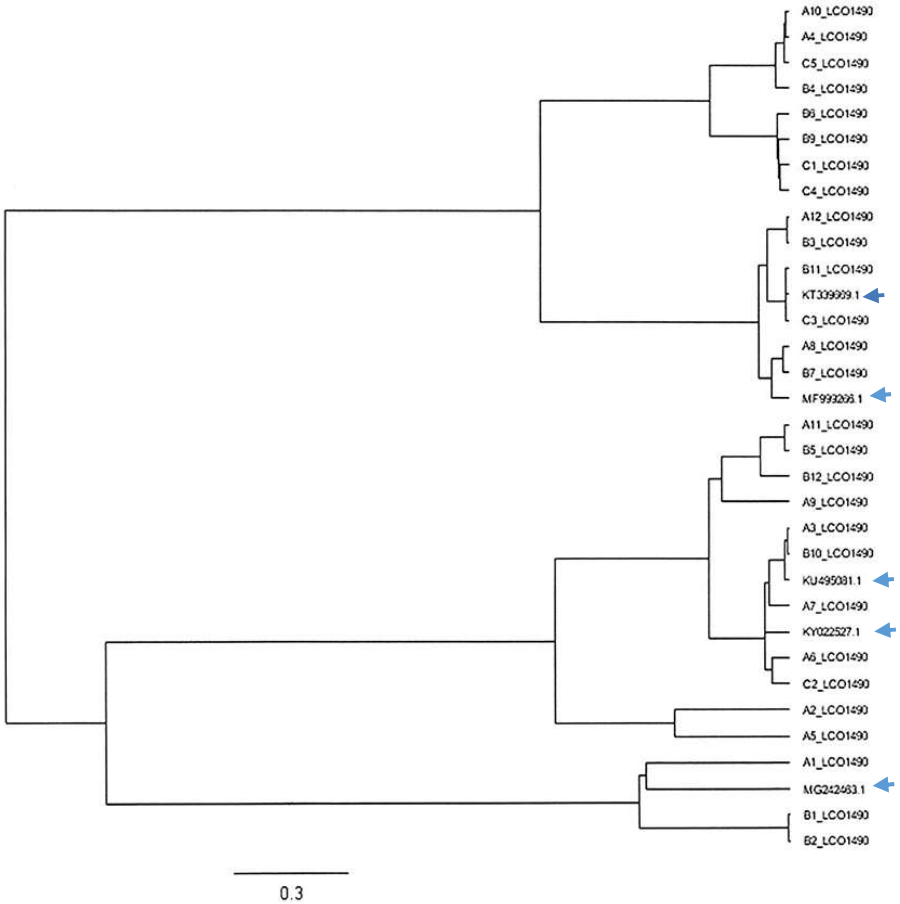


**Figure 4:** Phylogenetic analysis of 28 mitochondrial COX-I gene sequences of 28 mosquitoes specimens isolated from different western regions in Saudi Arabia with five *Aedes aegypti* samples selected from the GenBank entries using NCBI blast-n database. For phylogenetic analysis Beast2 program (https://www.beast2.org.com) and Fig tree V1.4.3 program were used. Blue arrows indicate the selected geneBank samples.

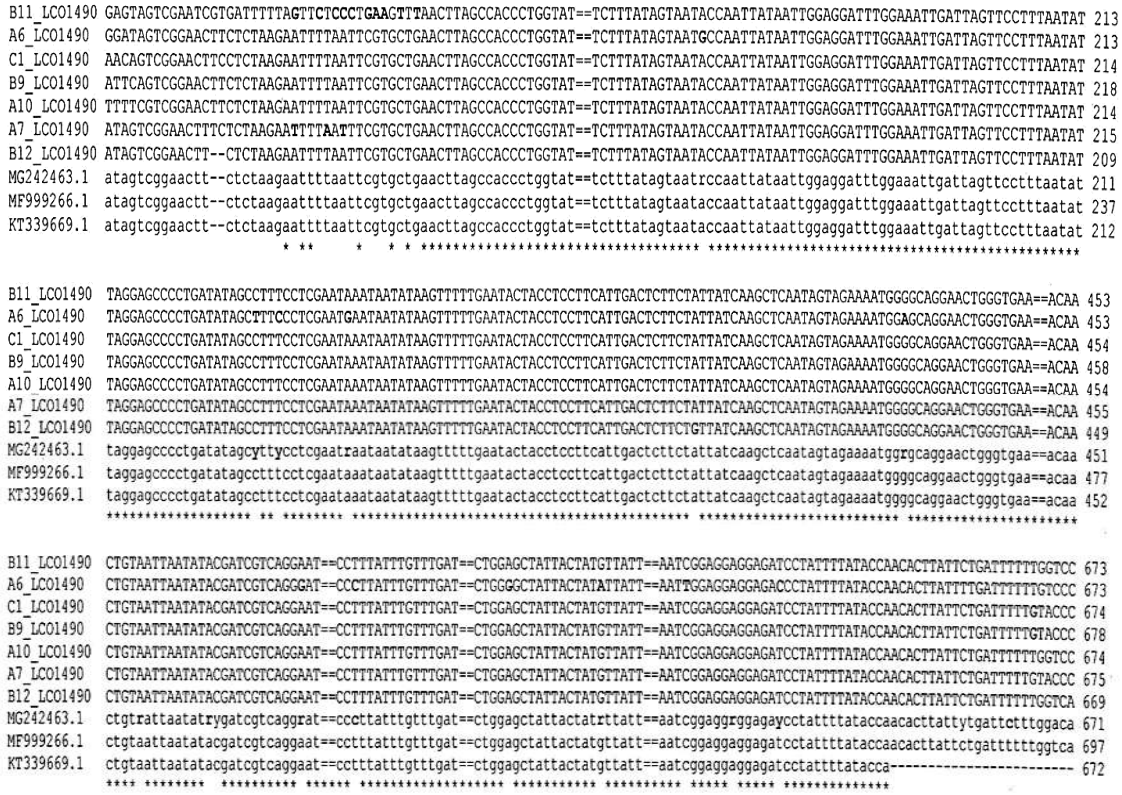

**Figure 5:** Multiple sequence alignment of seven mitochondrial COX-I sequences of *Aedes aegypti* specimens isolated from different western regions in Saudi Arabia, compare with the sequences of MG242463.1, MF999266.1 and KT339669.1 derived from GenBank. Sequence alignment was carried out in Clustal-O (1.2.4). "*"Indicates the absence of mutation between all samples ; (==) indicate long similar sequences in 10 samples.

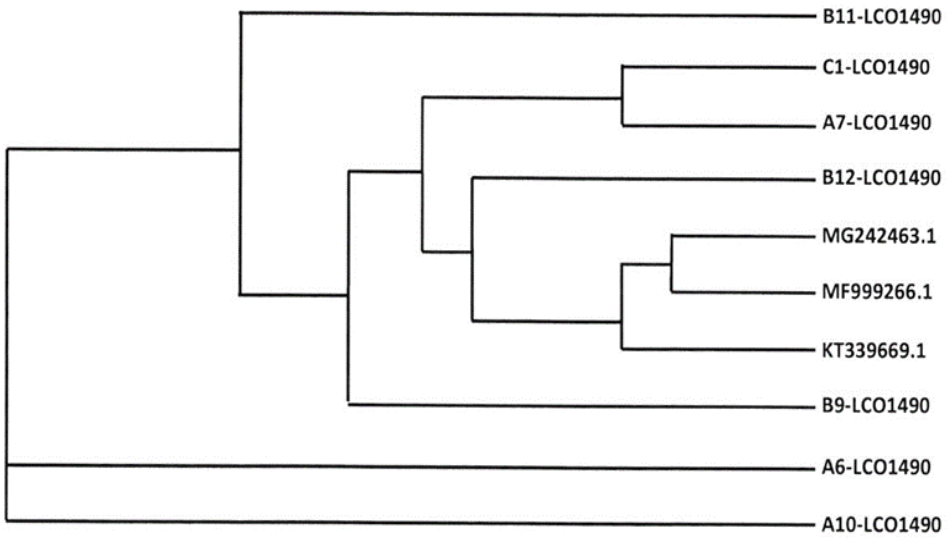


**Figure 6:** Phylogenetic analysis of mitochondrial COX-I gene sequences of seven *Aedes aegypti* specimens isolated from different western regions in Saudi Arabia. Noticeably, A7, B9,B12 and C1 are closely related to MG242463.1, MF999266.1 and KT339669.1. While A6, A10 and B11 emerged as separate out groups in the phylogenetic tree. For phylogenetic analysis, CLUSTAL-O (1.2.4) in the online website (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used.

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