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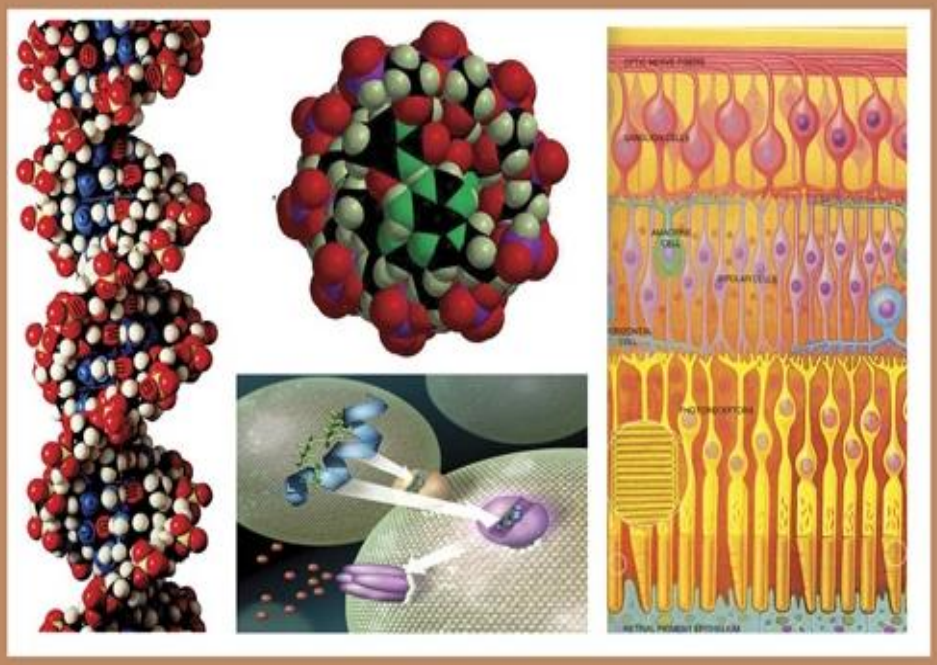

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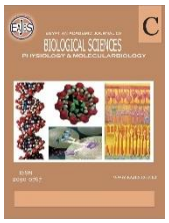

Persistence of *Aedes Aegypti* and Molecular Detection of DENV In Mosquitoes in Red Sea Governorate, Egypt

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ABSTRACT

Aedes aegypti (L), (Diptera: Culicidae) is a major vector for the spread of several dangerous arboviral diseases, including Dengue Fever. Dengue fever (DF) is one of the most common mosquito-borne viral zoonosis, affecting over 100 countries worldwide. Dengue fever (DF) and dengue haemorrhagic fever (DHF) are caused by four dengue viruses serotypes (DENV-1 to DENV-4). The purpose of this study was to determine the prevalence of *Ae. aegypti* mosquitos and their dengue virus carriers in Egypt's Red Sea governorate between 2019 and 2020. From September to December of 2019 and 2020, 3200 fourth larval instar mosquitoes and 1600 adult mosquitoes were collected and divided into 16 pools from 8 different regions associated with the Red Sea governorate. In addition to the standard morphological key, a molecular study was carried out using Cytochrome oxidase (COI) gene-specific primers. By using a PCR technique, all *Ae. aegypti* larvae and adults were tested for the presence of DENV. All pools collected from larvae and adults tested negative for DENV, indicating that, *Ae. aegypti* does not harbour DENV.

INTRODUCTION

Mosquitoes are the most common blood-sucking arthropods and important insect vectors of human disease, and they have influenced and continue to influence the course of human events. There are approximately 3500 mosquito species in the world, with *Anopheles*, *Aedes*, and *Culex* being the most important (Roberts and Janovy, 2009). MBDs (Mosquito-borne diseases) are rapidly spreading around the world. The rapid spread of highly aggressive pathogens, combined with resistance development in their vectors, results in fairly overwhelming epidemics and a significant challenge in modern parasitology and tropical medicine (Fernandes *et al.*, 2018 and Benelli, 2016). Involving simple overflow from enzootic, i.e., wildlife, cycles such as the West Nile virus reaching the Americas; secondary amplification in domesticated animals such as those of Japanese encephalitis, Venezuelan equine encephalitis, and Rift Valley fever viruses; and urbanization where humans suit the amplification hosts and peridomestic mosquitoes, primarily *Aedes aegypti*, act as a go-between human-to-human transmission in case of dengue, yellow fever, chikungunya, and Zika viruses. Chikungunya and Zika viruses are relatively new to the Western Hemisphere (Weaver *et al.*, 2018).

The yellow fever mosquito, *Ae. aegypti*, is responsible for the transmission of the most serious arboviral diseases, including dengue, chikungunya, and zika viruses (Kraemer *et al.,* 2015 and Souza-Neto *et al.,* 2019). This is a tropical and subtropical mosquito that is found all over the world but is native to the Sub-Saharan and African Sahelian regions, including Senegal, Cameroon, Kenya, Nigeria, Morocco, Western Sahara, Algeria, Tunisia, Egypt, and Sudan (Kamal *et al.,* 2018 and Kweka *et al.,* 2019). During the day, *Ae. aegypti* feeds on humans, rests at indoor locations, and breeds within and around the human environment, particularly in man-made containers (e.g., water jars, barrels, and tires) (Morrison *et al.,* 2008 and Scott and Takken, 2012).

Despite its name, *Ae. aegypti* was absent from Egypt for decades (Holstein, 1967), but it recently reappeared, causing a minor dengue outbreak in the Red Sea Governorate in 2017 (Abozeid *et al.,* 2018). Disease prevention is dependent on mosquito population control due to the lack of vaccines or antiviral treatments. As a result, it is critical to have knowledge of bionomics as well as the genetic structure of mosquitoes in terms of refractory or susceptible vector species (Urdaneta-Marquez and Failloux, 2011).

Identification of insects based on DNA barcoding has become a more efficient technique for species discrimination (Rolo, 2020), employing a small fragment of DNA that serves as a unique barcode for each species. This fragment corresponds to a ~ 650 base pair (bp) sequence found at the 50 ends of the cytochrome c oxidase subunit I gene (COI) in insects (Joyce *et al.,* 2018).

According to Tan *et al.,* (2011), dengue fever (DF) is one of the most serious mosquito-borne diseases affecting humans in terms of morbidity and mortality. Infected bites of female *Aedes* mosquitos, specifically *Ae. aegypti* (the primary vector transmitting the dengue virus in urban areas) transmit dengue fever to humans (WHO 2016 and Souza-Neto *et al.,* 2019). Horizontal (human-mosquito) transmission is the most well-known mode of DENV transmission. However, trans-ovarial/vertical transmission (Teo *et al.,* 2017) provides a mechanism for understanding how DENV persists in nature, i.e. in the absence of a host or in conditions unfavorable to its vector's activity (Martins *et al.,* 2012). The ability of *Aedes* mosquito eggs to survive for relatively long periods of time (even more than a year) allows the dengue virus to persist in the cold temperate, unfavorable environment for the adult vector (Brady *et al.,* 2014). Dengue fever is most commonly found in cities and suburbs, particularly in tropical and subtropical regions of the world (Fang *et al.,* 2021).

Molecular techniques have become an important diagnostic tool for viral infections, particularly because they allow for the specific determination of virus subtypes, which other methods do not. The most common method for detecting and quantifying dengue virus (DENV) is a reverse transcription (RT) followed by polymerase chain reaction (PCR) (De Paula *et al.,* 2001; Wang *et al.,* 2000 and Lanciotti *et al.,* 1992). These methods are quick and reliable, and they can be used early in the infection course to correctly identify the viral serotype (Fanson *et al.,* 2000). Several standardizations of RT–PCR for dengue virus detection have been described (Dettogni and Louro, 2012).

The current study is three-dimensional in nature, with the following goals: (1) morphological and molecular identification of the *Ae. Aegypti* mosquito using the COI gene; (2) identification of potential breeding habitats of the DENV vector; and (3) observation detection of DENV between 2019 and 2020 in the Red Sea governorate.

MATERIALS AND METHODS

Study Area, Larval, and Adult Collection Mosquitoes:

Study area: Mosquitoes were obtained from eight research locations in Egypt, which had DENV epidemics in 2017. A total of 16 pools of *Ae. aegypti* larvae and adults mosquitos were collected from different regions in Al-

Bahr Al-Ahmar, Red sea governorate, Egypt between September to December over two consecutive years (2019 and 2020), including Safaga (Safaga and Industrial Area), Al-Qusayr (Algarf, Owaina and New Owaina) and Al-Ghardaqah (Altaqwaa, Alarab, Mujahid and Almilaha) (Fig. 1).

Larval collection: Standard mosquito larval surveys were conducted during field surveillance collecting by inspecting all indoor and outdoor water containers in all regions indicated above. Mosquito larvae were obtained using fine-mesh fishnets from both indoor and outdoor containers. Outdoor larval surveys were undertaken within a 15-meter radius of residences, Wongkoon, *et al.*, (2007). *Aedes* immatures in their third and fourth larval instars were tested from all water containers. Water was poured into the fishnet from very tiny containers. By immersing the net in the liquid and swirling it from top to bottom, massive water containers were tested, sampling all sides of the container Wongkoon et al. (2007). Immediately after collection, mosquito larvae were placed in plastic bags filled with water from the water container until further processing.

To detect *Ae. aegypti* mosquito breeding areas, including any readily accessible water containers, both natural and artificial, were inspected in and around houses. This research included 12 container classifications. Indoor vessels included huge and tiny water tanks, plastic tanks, and cement tanks. Outdoor containers included small and big water tanks, plastic tanks, cement tanks, used tires and cans, animal pans, and plastic bottles. For the water jar, we categorized water jars into two categories: small water jars (less than 100 L) and big water jars (more than 100 L). The 12 containers were divided into two categories: water storage and trash.

Adult collection: Adult mosquitoes were gathered using CDC light traps (Bioquip, USA). Each CDC light trap was operated once overnight weekly throughout the study period. The collected mosquitoes were packaged, labeled, and conveyed to the insectary of Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt for morphological and molecular identification, and some of the collected samples were kept at -80 ℃ for detection of DENV.

Fig. 1: The map depicted the geographical distribution of mosquitos collected in this study from Egypt's Red Sea governorate in 2019 and 2020.

Morphological identification of *Ae. Aegypti* (L):

At the General Organization for Institutes and Teaching Hospitals, Ministry of Health, Research Institute of Medical Entomology, Dokki, Giza, Egypt, fourth larval instars and adult mosquitoes were morphologically identified using taxonomic

keys according to (Mattingly and Knight, 1956; Harbach, 1985 and Soltani *et al.*, 2017).

Using a sterile mortar and pestle, the collected mosquito larvae and adults were ground to approximately 400 larvae and 200 adult mosquitoes in phosphate-buffered saline (PBS). The tissue homogenate was centrifuged at 3000 rpm for 10 minutes before the supernatant fluid was frozen at -80°C for further DNA and RNA extraction.

Molecular Identification:

DNA Extraction:

DNA extraction was carried out at the Animal Health Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt, according to the manufacturer's instructions, using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Cat. no. K0721). The Nanodrop Qubit 3.0 Fluorometer was used to assess the quantity and quality of DNA in two extracted samples (larvae and adults).

PCR Amplification and DNA Sequencing:

Amplification by Cytochrome Oxidase I (COI) was performed in a T100 thermocycler (BioRad, Hercules, California, USA) according to Folmer *et al*., 1994, and 'in T100 thermocycler (BioRad, Hercules, California, USA). The PCR reaction mixture was adjusted to 50 µl and contained 25 µl of Applied Biosystems™, AmpliTaq Gold® 360 Master Mix (Thermo Fisher Scientific, USA, Cat. No. 4398876), 1 µl of forwarding primer (LCO1490-F 5' -GGT CAA CAA ATC ATA AAG ATA TTG G- 3'), 1 µl of reverse primer (LCO1490-R 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA- 3), 8 µl of extracted DNA and finally complete to 50 µl nuclease-free water. The following changes were made to the PCR reaction conditions: An initial denaturation at 95°C for 5 minutes was followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 51°C for 1 minute and extension at 72°C for 1 minute., followed by a final extension at 72°C for 10 minutes, Kumar *et al.*, (2007).

In comparison to the 50 bp DNA Ladder RTU (GeneDirex, cat. no. DM101-0100), the PCR product was visualized using the Imager Gel Doc™ XR+ Imaging system (BIO-RAD) and Image lab™ software for gel image analysis. The PCR product was purified using the QIAquick® Gel Extraction Kit (QIAGEN, USA, Cat. no. 28704) and sequenced using the BigDye® Terminator v3. and cycle sequencing kit (Applied Biosystems, USA), as directed by the manufacturer.

This study's COI sequence was deposited in GenBank under the accession number MT328866 (http://www.ncbi.nlm.nih.gov). The GenBank and BOLD databases were searched for mosquito identification using the BLAST similarity search (available at http://www.ncbi.nlm.nih. gov) (National Center for Biotechnology Information, Rockville Pike, Bethesda, MD).

Phylogenetic Analyses:

The phylogenetic tree was built using a total of 17 COI sequences, including 16 sequences downloaded from GenBank in addition to the sequence obtained in the current study. The tree was constructed using a Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree, which was inferred from 1000 bootstrap replicates, was created using a MEGAX (Kumar *et al*., 2018).

Molecular Detection of Dengue Virus (DENV):

RNA Extraction:

RNA was extracted from larval and adult stages of collected mosquitos using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany; cat. No. 52904) according to the manufacturer’s instructions throughout 2019 and 2020. The OD260/OD280 spectrophotometer (BIO-RAD, USA) was used to determine the purity of the RNA.

Reverse Transcription–Polymerase Chain Reaction (RT–PCR):

Thermo Scientific Verso 1-Step RT-PCR Reddy Mix Kit, Cat. No. #AB-1454/LD/A, was used for first-strand cDNA. Lanciotti, *et al.*, 1992, used first-strand cDNA and primers DC1 and DC2, DC1: (5′-TCAATATGCTGAAACGCGCGAGAAAC CG-3′), DC2: (5′-TTGCACCAACAG TCAA

TGTCTTCAGGTTC-3′),**.** The PCR reaction mixture was adjusted to 50 µl and contained 0.5 µl Verso Enzyme Mix, 12.5 µl 2x 1-step PCR Reddy Mix, 1.2 µl RT Enhancer, 2 µl Forward primer (20 pmoles), 2 µl Reverse primer (20 pmoles), 7 µl Template RNA and 24.8 µl highly pure, nuclease-free water for use in all molecular biology applications, 1000 ml, Cat. No. 129115, Qiagen, USA. The following changes were made to the PCR reaction conditions: cDNA synthesis at 50°C for 15 minutes and Verso inactivation, 95°C for 5 minutes of 1 cycle, followed by 40 cycles of denaturation at 95°C for 55 seconds, annealing at 51°C for 55 seconds, extension at 72°C for 1.5 minutes, and final extension at 72°C for 10 minutes.

A BIO-RAD® PCR system T100 thermocycler (BioRad, Hercules, California, USA) was used for DNA amplification. The amplified PCR products were separated using 1.5% agarose gel electrophoresis. In comparison to the 100 bp DNA ladder RTU, the Imager Gel Doc™ XR+ Imaging system (BIO-RAD) and Image lab™ software for gel image analysis outperformed the DNA band of the predicted size (GeneDirex, Cat. No. DM101-0100).

RESULTS

Mosquito Identification and Distribution of DENV vector, *Ae. aegypti* larvae and Adults in Red Sea Governorate:

In this study, we collected 3200 mosquito larvae from 8 different locations in the Red Sea governorate between September to December 2019 and 2020 (Table 1). Table 2, depicts the larval breeding habitats of *Ae. aegypti* mosquitoes. According to our findings, water storage, particularly water jars, cement and plastic tanks served as primary breeding habitats for *Ae. aegypti* mosquitoes. Trash containers, on the other hand, are regarded as minor breeding sites for *Ae. aegypti* in this study. Of the 3200 mosquito larvae collected, 2140 (66.9%) were collected outdoors, while 1060 (33.1%) were collected indoors, (Table 2).

Male *Ae. aegypti* mosquitos were collected at a higher rate than females during the study period, (Table 3).

In the insectary of the Zoology and Entomology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt, 3200 larvae (100%) were identified as *Ae. aegypti* based on the morphology of the comb scales and cephalic setae, which are single and large teeth at the base of thoracic setae 11M and 11T, (Fig. 2).

Between 2019 and 2020, a total of 1600 female mosquitoes were collected from the same eight locations where mosquito larvae were collected in the Red Sea governorate between September to December. Of the 1600 female mosquitoes collected, 1220 (76.25%) were found outdoors and 380 (23.75%) were found indoors, (Table 4).

Table 1: *Aedes* mosquito sampling areas visited for larvae and adults in the Red Sea governorate in 2019 and 2020.

S. No	Site		*Aedes aegypti* (2019)		*Aedes aegypti* (2020)	
			Adults (n= 900)	Larvae (n= 2050)	Adults (n= 700)	Larvae (n= 1150)
1.	Safaga	Safaga	140 (15.5%)	160 (7.8%)	60 (8.6%)	240 (20.9%)
2.		Industrial Area	110 (12.2%)	205 (10%)	90 (12.9%)	195 (17%)
3.	Al-Qusayr	Algarf	90 (10%)	280 (13.7%)	110 (15.7%)	120 (10.4%)
4.		Owaina	150 (16.7%)	310 (15.1%)	50 (7.1%)	90 (7.8%)
5.	Al-Ghardaqah	Altaqwaa	85 (9.4%)	250 (12.2%)	115 (16.4%)	150 (13%)
6.		Alarab	70 (7.8%)	230 (11.2%)	130 (18.6%)	170 (14.8%)
7.		Mujahid	125 (14%)	305 (14.9%)	75 (10.7%)	95 (8.3%)
8.		Almilaha	130 (14.4%)	310 (15.1%)	70 (10%)	90 (7.8%)

Table 2: Shows the number and percentage of *Aedes aegypti* larvae collected indoors and outdoors from various natural and man-made containers in the Red Sea governorate, Egypt.

S. No	Habitats			*Aedes aegypti* (2019)		*Aedes aegypti* (2020)	
				Larvae (n= 2050)		Larvae (n= 1150)	
1.	Water storage	Indoor (%)	Large water tanks	34.9 %	250 (12.2%)	30 %	95 (8.3%)
2.			Small water tanks		180 (8.8%)		50 (4.3%)
3.			Cement tanks		155 (7.6%)		105 (9.1%)
4.			Plastic tanks		130 (6.3%)		95 (8.3%)
5.		Outdoor (%)	Large water tanks	65.1 %	465 (22.7%)	70 %	300 (26.1%)
6.			Small water tanks		245 (11.9%)		125 (10.9%)
7.			Cement tanks		195 (9.5%)		110 (9.6%)
8.			Plastic tanks		180 (8.9%)		85 (7.4%)
9.			Animal pans		55 (2.7%)		25 (2.2%)
10.	Trash		Used tires		70 (3.4%)		75 (6.5%)
11.			Plastic bottles		100 (4.8%)		65 (5.6%)
12.			Used cans		25 (1.2%)		20 (1.7 %)

***S. No= Sample number**

Table 3: Shows the locations of Female and male *Aedes* mosquito sampling sites in the Red Sea governorate in 2019 and 2020.

S. No.	Site		*Aedes aegypti* (2019)		*Aedes aegypti* (2020)	
			Adults (female) (n= 900)	Adults (male) (n= 1300)	Adults (female) (n= 700)	Adults (male) (n= 1050)
1.	Safaga	Safaga	140 (15.5%)	180 (13.8%)	60 (8.6%)	120 (11.4%)
2.		Industrial Area	110 (12.2%)	155 (11.9%)	90 (12.9%)	135 (12.9%)
3.	Al-Qusayr	Algarf	90 (10%)	130 (10%)	110 (15.7%)	150 (14.3%)
4.		Owaina	150 (16.7%)	240 (18.5%)	50 (7.1%)	95 (9%)
5.	Al-Ghardaqah	Altaqwaa	85 (9.4%)	135 (10.4%)	115 (16.4%)	155 (14.8%)
6.		Alarab	70 (7.8%)	115 (8.8%)	130 (18.6%)	175 (16.7%)
7.		Mujahid	125 (14%)	170 (13.1%)	75 (10.7%)	115 (10.9%)
8.		Almilaha	130 (14.4%)	175 (13.5%)	70 (10%)	105 (10%)

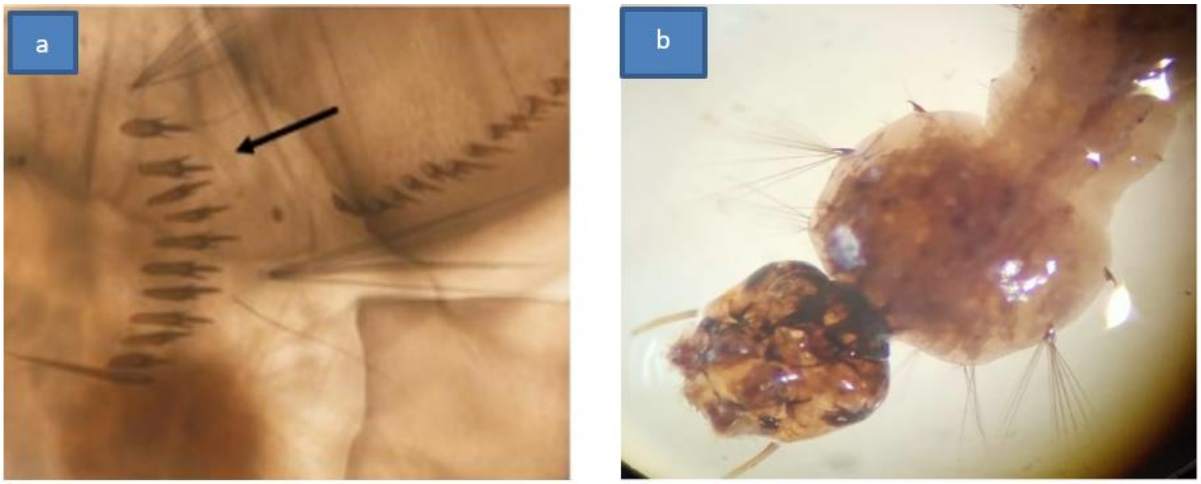

Fig. 2: Identification of *Aedes aegypti* larvae using microscopy, (a) pitchfork-shaped comb scales in one row with a distinct middle denticle and lateral denticles indicated by a black arrow (200X magnification). (b) Ventral view of the head and thorax.

Table 4: Counts and percentage of female *Ae. aegypti* collected indoors and outdoors in Egypt's Red Sea governorate in 2019 and 2022.

S. No	Site	*Aedes aegypti* (2019)	*Aedes aegypti* (2020)
		Adults (n= 900)	Adults (n= 700)
1.	Indoor	200 (22.2%)	180 (25.7%)
2.	Outdoor	700 (77.8%)	520 (74.3%)

* S. No= Sample number

Based on the morphology of the terminal part of the abdomen is needle-shaped, all the tibiae are dark anteriorly, the fore and mid tarsi have a white basal band on tarsomeres I and II, the hind tarsus has a broad basal white band on tarsomeres I–IV, and tarsomere V is all white, a total of 1600 female mosquitoes (100%) were identified as *Ae. aegypti*. The white lyre shape on the dorsal side of the thorax distinguishes this species from others in the genus, (Becker *et al.*, 2010), (Fig. 3).

The DNA sequence of a cloned PCR product of DENV vector identification:

The PCR was performed initially on 50 random *Ae. aegypti* DNA samples from larvae and adults using primers for the COI DNA partial gene, and a PCR product of 678bp was obtained (Fig. 4).

The resulting sequence was identical to all other *Ae. aegypti* sequences in GenBank. The species identified and collected in this study could thus be specified based on their COI gene, resulting in 100% compatibility between molecular and taxonomic identification, indicating that the COI barcode is a useful tool to supplement taxonomy for mosquito species identification (Fig. 5)

(a) (b)

Fig. 3: Female *Aedes aegypti* morphological identification, **a:** The scutum is mostly covered in narrow dark brown scales with a distinct pattern of light scales (lyre shape). **b:** There is a patch of broad white scales and some dark and pale narrow scales on the upper part of the postpronotum.

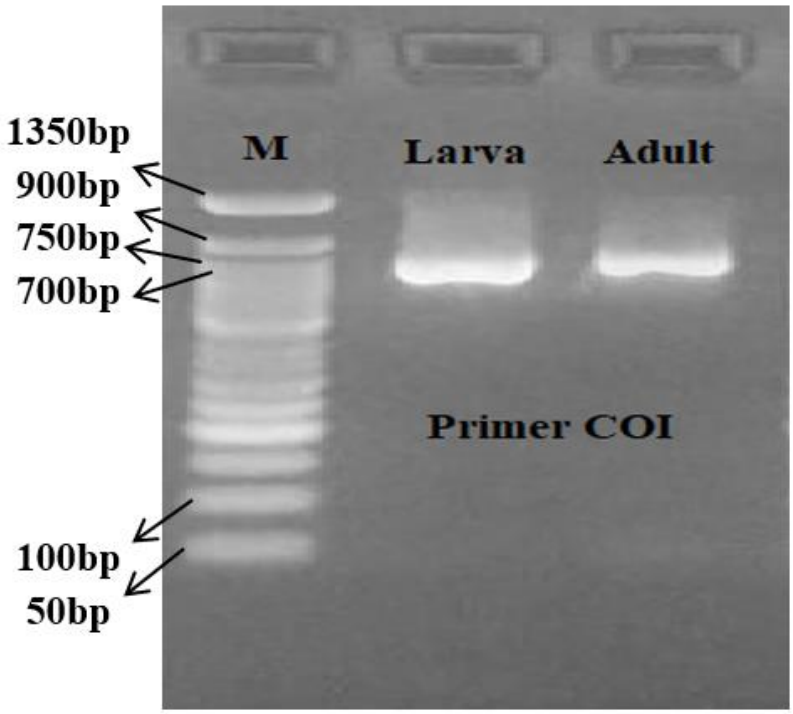

Fig. 4: Amplification of the COI region of *Ae. aegypti* collected. Lane 1(M): represents a marker 50 bp, Lane 2: represents *Ae. aegypti* larvae and Lane 3: represents *Ae. aegypti* adults.

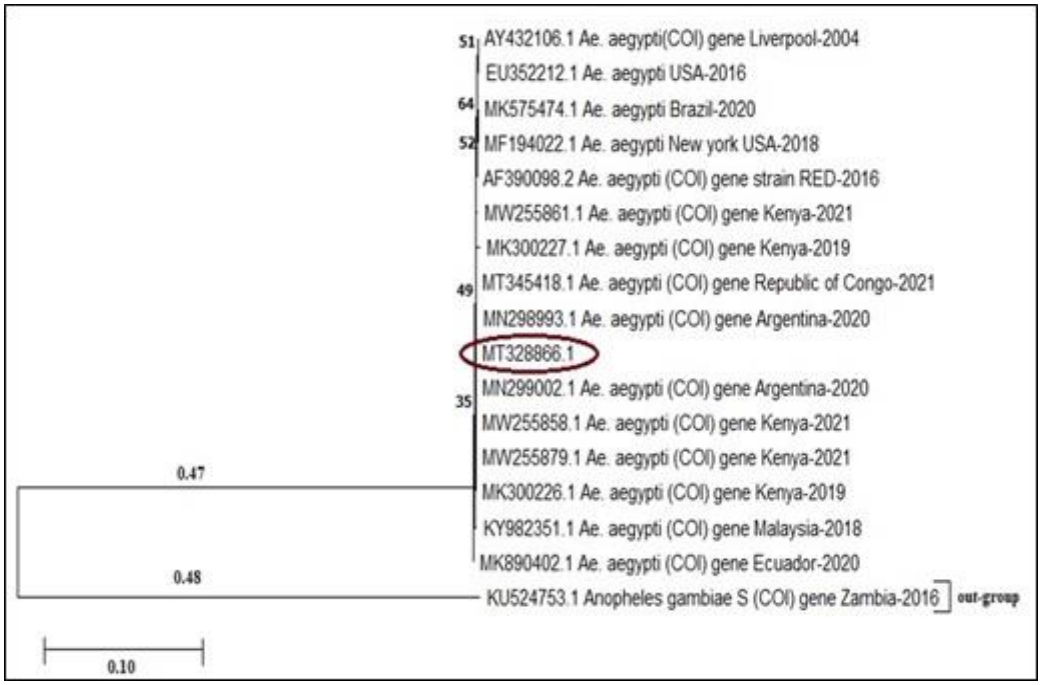

Fig. (5): In Egypt, a phylogenetic tree of *Ae. aegypti* was constructed using the Maximum Composite Likelihood method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches (1000 replicates). The *Anopheles gambiae* S (COI) sequence was used as an outgroup.

DENV Prevalence in *Ae. aegypti* Mosquitoes:

from 16 pools of 3200 fourth larval instar and 1600 adult mosquitoes collected in 2019 and 2020 from September to December from 8 different regions associated with the Red Sea governorate (each pool contained 400 larvae and 200 adults mosquitoes) from various locations described above, all pools were negative (**Fig. 6**). Positive control for DENV serotypes was available beginning with the 2018 dengue in Vacsera, Dokki, Giza, Egypt. The agarose gel electrophoresis of PCR for the dengue virus is depicted in this figure. After amplification with universal dengue primers, the correct size of the DNA product (480 bp) was obtained as a positive control.

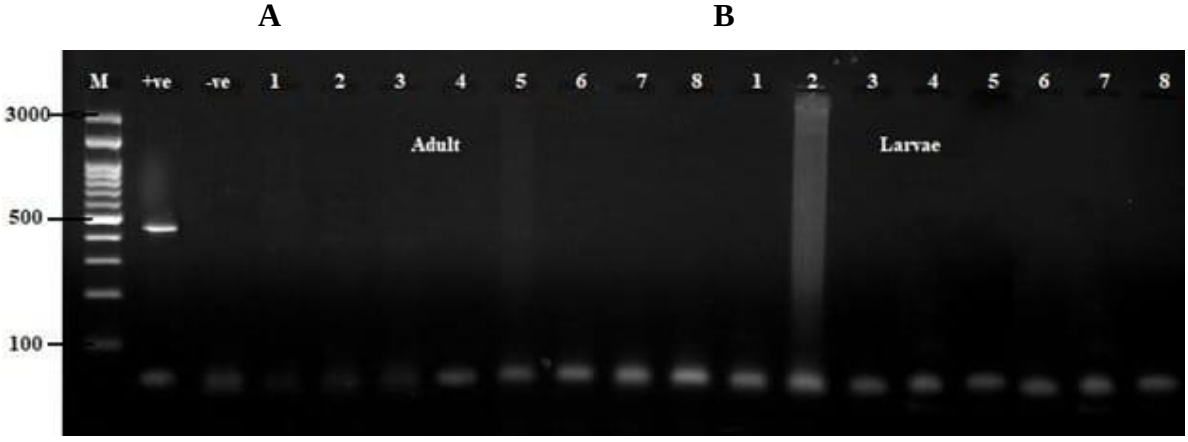

Fig. (5): Electrophoresis of PCR products on 1.5% agarose gels for the detection of dengue virus in pools of *Ae. aegypti* mosquitos. Lane M: represents a marker; Lane +ve: represents a positive control; Lane –ve: represents a negative control; **(A):** lanes 1-8 represents a negative PCR product for *Ae. aegypti*, adults; **(B):** lanes 1-8 negative PCR product for Larvae of *Ae. aegypti.*

DISCUSSION

The current study found *Ae. aegypti* in the Red Sea governorate, and *Ae. aegypti* was recovered from water sources, with a few adult females trapped indoors and outdoors. In endemic areas, *Ae. aegypti* is closely associated with human environments, such as indoor and outdoor artificial containers such as small water tanks, large water tanks, cement tanks, plastic tanks, used cans, used tires, plastic bottles, and animal pans. For larval development, we divided water jars into two categories: small water jars (<100 L) and large water jars (>100 L), (Burkot *et al.*, 2007). These species' larvae were discovered in clear and clean water in a variety of artificial and natural containers (Rattanarithikul and Panthusiri, 1994). Similar findings were obtained by (Chareonviriyaphap *et al.*, 2003).

In Egypt, *Aedes* species were reported by Kirkpatrick (1925), Gad (1963) (*Ae. aegypti, Ae. caspius and Ae. detritus*). Holstein, (1967) reported a complete eradication of *Ae. aegypti* from Egypt. According to Mostafa *et al.* (2002), *Ae. detritus* was found in several governorates (Asyut, El Fayium, Giza, Aswan, El Wady El Gadeed, and South Sinai). *Ae. caspius* was discovered in Asyut and Aswan, as well as Kena and El Wady El Gadeed as larvae. Morsy *et al.* (2003, 2004) found *Ae. caspius* in Qalyoubia, Giza and Greater Cairo. Shaalan *et al.* (2005a, 2005b) found *Ae. aegypti* in water sources in Aswan. Mikhail *et al.* (2009) reported the presence of *Ae. caspius* and *Ae. detritus in* Cairo, Sharkia, Qualyoubia, and Giza. Abdel-Hamid *et al.* (2011) reported *Ae. (O.) caspius* and *Ae. (O.) detritus* in El Menoufia. Abozeid *et al.* (2018) reported *Ae. aegypti* in the Red Sea governorate. Males were more prevalent than females in this research, which is consistent with Eldigail *et al.*, 2018, Koh *et al.*, 2008, Hussen et al. 2020, but in opposition to the observation of Nava-Aguilera *et al.* (2017).

Another study (Murray *et al.* 2013) showed that higher temperatures (>25°C) resulted in a greater number of mosquitoes with a proclivity for blood eating. Additionally, it has been proven that a temperature increase of 1 °C (above normal) increase the probability of dengue transmission by 1.95 times (Sang *et al.*, 2014). Rainfall (humidity) is another ecological component that creates a perfect breeding environment for mosquitos, resulting in increasing their population density. Furthermore, people often remain inside during the rainy season, increasing the likelihood of *Ae. aegypti* (particularly) coming into touch with humans. Therefore, the inside stays of *Ae. aegypti* and humans during the monsoon season give an optimal chance for DENV to be transmitted/communicated. For this reason, in the present study, we collected the mosquitoes from September to December in 2019 and 2020. Another study has also revealed that the egg viability (Rahman *et al.*, 2010) and population size of the vector (Micieli and Campos, 2003) increase in humid conditions.

DF is caused by four DENV serotypes (DENV 1–DENV 4), members of the Flavivirus genus and family Flaviviridae. These serotypes are transmissible to hosts by *Ae. albopictus* and *Ae. aegypti*. Adult female *Aedes* acquire the virus after biting an infected individual during the viral phase and spread it via bites to uninfected persons (Sharma *et al.*, 2014). In the previous five years, two DF outbreaks have occurred in Upper Egypt, notably in El Quseir, Red Sea Governorate (2017) and Dairot District, Asyut Governorate (2015), where *Aedes* was discovered to be predominant. To our knowledge, there is presently no epidemiological data on the disease in the Red Sea governorate. This study attempted to screen new possibly endemic areas and check the epidemiological state of the previously infected areas. As a result, we determined the absence of DENV in *Ae. aegypti* by screening larvae and adults obtained from the Red Sea governorate. The relevant data was recorded through the Hurghada Health Directorate. In this study, all samples collected during 2019

and 2020 were negative. A thorough review of the literature using a variety of methodologies found a dearth of current epidemiological data on the frequency and risk factors for DF in Egypt.

In parallel with screening for the DF prevalence, dengue outbreak investigations (2019 and 2020) revealed a high level of vector infestation in natural and man-made water-holding containers in human dwellings as well as in public areas, particularly during September, October, November and December. In light of this research, we strongly advise avoiding DF infection by improving water-storage practices (such as the proper covering of water-holding containers to prevent vector breeding and personal protective measures, especially during the rainy season to prevent vector human contact and disease incidence. Additionally, removing unnecessary containers and properly sealing water reservoirs are important in preventing females from dispersing outdoors.

Conclusion

The present study is the first report of *Ae. aegypti* identification from the Red Sea Governorate in Egypt during 2019 and 2020. Sequence analysis of COI from morphologically identified *Aedes* confirmed the identity of the investigated species. DFV detection from collected *Ae. Aegypti* revealed its absence from the vector mosquito, raising questions for further studies about the possibility of DFV detection in other areas.

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