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Distribution of mosquitoes and the first record of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Diptera: Culicidae) in the Riyadh Province, Kingdom of Saudi Arabia

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Abstract
Mosquitoes are among the most important vectors that transmit several parasitic and viral diseases in different regions of the world. Mosquito surveillance was carried out from 2012 to 2017 to determine the occurrence and distribution of mosquito species in five places in the Riyadh Province (Riyadh city, Al-Kharj, Ad Dilam, Al Majma'ah, and Al Duwadimi) in the Kingdom of Saudi Arabia (KSA). Larval collections were carried out using standard dippers and adult collections with Bio traps. The mosquito collection comprised *Anopheles dthali*, *An. pretoriensis*, *Culex pipiens*, *Cx. quinquefasciatus*, and *Aedes aegypti*. The presence of *Ae aegypti* is considered to be the first record in the mentioned places. The occurrence of *Ae. aegypti* represents a serious risk of transmission of *Ae. aegypti*-borne diseases in the region. Therefore, active entomological surveillance and proper vector control are needed to prevent the further spread of this vector and the transmission of *Ae. aegypti*-borne diseases in the region.

Keywords: mosquitoes, *Aedes aegypti*, first record, Riyadh province, KSA

1. Introduction
Mosquito-borne diseases (MBD) such as malaria and dengue fever are endemic in the southwestern and western regions of the Kingdom of Saudi Arabia (KSA), respectively [1,2]. During the last three decades, the KSA had s witnessed major epidemics of mosquito arboviral diseases which were Rift Valley fever (RVF), and dengue fever (DF). The outbreak of RVF occurred from August 2000 to September 2001in the southern regions of KSA and resulted in 1500 infected human cases and 215 deaths [3]. Furthermore, several outbreaks of DF were reported from the western coastal region in the country [4]. The first documented outbreak of DF in KSA was in 1994 in Jeddah, where 289 confirmed cases were recorded [5]. Thereafter, sporadic outbreaks of DF have been reported in the western region of the KSA [6,7]. More recently, local transmission of DF in the KSA has been reported in different cities including Jeddah, Makkah, Al Madinah, Jazan, AL Taif, and Najran [8, 9, 10].
Mosquitoes comprise the most important vectors of human diseases in the Kingdom of Saudi Arabia (KSA). Studies on the fauna and distribution of mosquitoes in the country revealed about 49 species, that include 18 anophelines and 31 culicines [11]. Among these species, *Anopheles arabiensis*, *An. sergentii* and *An. stephensi* are currently considered as the potential vectors of malaria parasites [11, 12], *Culex pipiens*, and *Cx. quinquefasciatus*, probable vectors of human filariasis and West Nile virus [13], *Cx. tritaeniorhynchus* and *Aedes vexans arabiensis* are proposed to be vectors of RVF in KSA [14, 15], *Aedes aegypti* the main vector of dengue fever in the country [16]. Most of these mosquito species are recorded in almost all surveyed regions in the Eastern [17], Western [18, 19], and Southern areas of KSA [20, 21], including the Riyadh Region [22]. However, *Aedes agyepti* was recorded only in the western and southern regions of the country [19, 20]. Hence, studies on the identification, fauna, and distribution of vector species are important requirements for disease control.

The spread of invasive mosquito species into new areas is highly problematic because it would introduce some vital viral diseases (e.g. Dengue fever, Chikungunya, yellow fever, Zika virus) into these areas. *Aedes* (Stegomyia) *aegypti* (Linnaeus, 1762) is one of the most important invasive mosquito species that has expanded its habitats tropics and subtropics of the world [23]. *Aedes aegypti* the primary vector of dengue fever, is widely distributed in the southern and western regions of the KSA [19, 20, 21]. Although studies on mosquito fauna were previously conducted in the Central region of KSA including the Riyadh region, *Ae. aegypti* has not been recorded yet in these areas [22]. Monitoring and updating fauna and their distribution in such regions are important for the control of the mosquito-borne disease program. Therefore, entomological surveillance has been conducted throughout the Riyadh city, Al-Kharj, Ad Dilam, Al Majma'ah, and Al Duwadimi that belong to the Riyadh Province in KSA to determine mosquito fauna, their spatial distribution, and to investigate whether the exotic mosquitoes especially *Ae. aegypti* has infested the region. Here, the first record of *Ae. aegypti* in the Riyadh Province in KSA is documented.

2. Materials and Methods

2.1. Study area

The Riyadh Province is located in the central region of the country (23° 0′ 0″ N, 45° 30′ 0″), at 600 meters above sea level, and consists of different cities including Riyadh city, Al-Kharj, Ad Dilam, Al Majma'ah, Al Duwadimi, etc... Riyadh city is the largest city in the Riyadh Province and it is also the national capital of the KSA. The Province covers an area of 404,240 km² with a population of 8,216,284 people and it is bordered by the provinces of Al Qassim from the North, Najran from the south, Al Madinah, Makkah, and Asir from the west, and Ash Sharqiyah from the east. The temperature in the Riyadh Province varies from 14.5° to C -30 °C in winter with some very cold nights (i.e. approach freezing temperature) and 44 °C to 47 °C in summer. The annual precipitation not exceeding 100 mm and the rainfall occurs from November to April with the wettest days during April.

2.2. Study sites

Mosquito surveillance was conducted twice a year (September - October, and November) from 2012 to 2017 in different areas located in the Riyadh Province namely'; Riyadh city, Al-Kharj, Ad Dilam, Al Majma'ah, and Al Duwadimi as illustrated in figure 1. The surveys were conducted in the months of moderate temperatures (September -November) during which the mosquito densities are consistently higher than in months of the summer and or winter. in the Riyadh Province. The surveys were conducted by the team of the General Directorate of Vector-Borne and Zoonotic Diseases, the Saudi Ministry of Health in Riyadh city. The mosquito adult collections were done in the four governorates; Al-Kharj, Ad Dilam, Al Majma'ah, and Al Duwadimi; whereas. larval surveys were conducted in the governorates of Al-Kharj and Ad Dilam only. In Riyadh city, 18 areas were surveyed for mosquitoes; where the adult and larval collections were done in 8 and 17 of these areas, respectively. The detailed information of the surveyed sites is presented in table 1. Search for adult mosquitoes was done in houses, farms, labor and guardrooms, health centers, schools under construction, and buildings under construction and workshops. The larval surveys were done in farms, tires, water pools, buildings under construction (e.g. houses, schools, etc…), birds drinking water containers, leaking pipes, water air-conditioner, water basin for ablution in a mosque, a basement of buildings, surface water, water cooler, and rain filled-pools in valleys.

2.3. Mosquito surveillance

2.3.1. Adult collection

Adult mosquito collection was done in the Riyadh Province from 2012 to 2017. The adult mosquitoes were collected using V-Mart Super photo-catalyst Black Hole (BH) traps (BLACK HOLE mosquito trap tipe Bio Trap (perangkap nyamuk) (original KOREA). In each collection site, six traps were deployed to collect adult mosquitoes (3 indoors and 3 outdoors). These traps were connected to a power source before sunset and collected the next morning just after sunrise. The captured adult mosquitoes by these traps were collected every day, sucked up using standard mosquito mouth aspirators, and transferred into well-labeled papers covered at the top with a mosquito mesh fixed with plastic rubber. The paper cups containing adult mosquitoes were then kept in large polystyrene boxes lined at the bottom with relatively wetted paper towels, and transported to the Laboratory of the Directorate of Vector-Borne and Zoonotic Diseases at the Ministry of Health in Riyadh city. In the laboratory, the field-collected adult mosquitoes were kept fresh for subsequent identification to species level.

Table 1: The surveyed areas for mosquitoes in the Riyadh Province, Saudi Arabia.

Governorate/City	Area	Coordinates		Collection	
				Adult	Larvae
Ad Dilam		47°05'53.2"E	24°01'32.8"N		
Al-Kharj		47°13'25.8"E	24°05'16.6"N		
Al Majma'ah		45°20'10.5"E	25°54'47.0"N		
Al Duwadimi		44°26'00.6"E	24°30'14.6"N		
Riyadh city	Al Malaz	46°44'27.1"E	24°40'15.0"N		
	Al Sina'iyah	46°44'37.5"E	24°39'09.7"N		
	Irqah	46°35'35.8"E	24°40'41.0"N		
	Umm Al Hamam	46°38'48.4"E	24°41'33.8"N		
	Al Driyiah	46°31'47.6"E	24°45'02.8"N		
	Ad dar Albaida	46°46'55.2"E	24°34'17.5"N		
	Wadi Hanifa	46°42'20.3"E	24°35'32.9"N		
	Badr	46°42'22.8"E	24°33'34.0"N		
	Ash Shifa	46°41'14.8"E	24°33'59.6"N		

	Al Hazm	46°39'07.0"E	24°32'14.7"N		
	Al Mursalat	46°41'05.3"E	24°45'05.5"N		
	Al Masiaf	46°40'56.2"E	24°45'26.5"N		
	Al Gadir	46°39'20.5"E	24°46'33.3"N		
	Ar Rabi	46°39'46.1"E	24°47'19.4"N		
	Alyasmin	46°38'18.3"E	24°49'19.2"N		
	Al Khuzama	46°36'19.7"E	24°42'47.5"N		
	Al Amaaria	46°25'20.6"E	24°48'11.2"N		
	Al Malaqa	46°37'00.7"E	24°48'14.7"N		

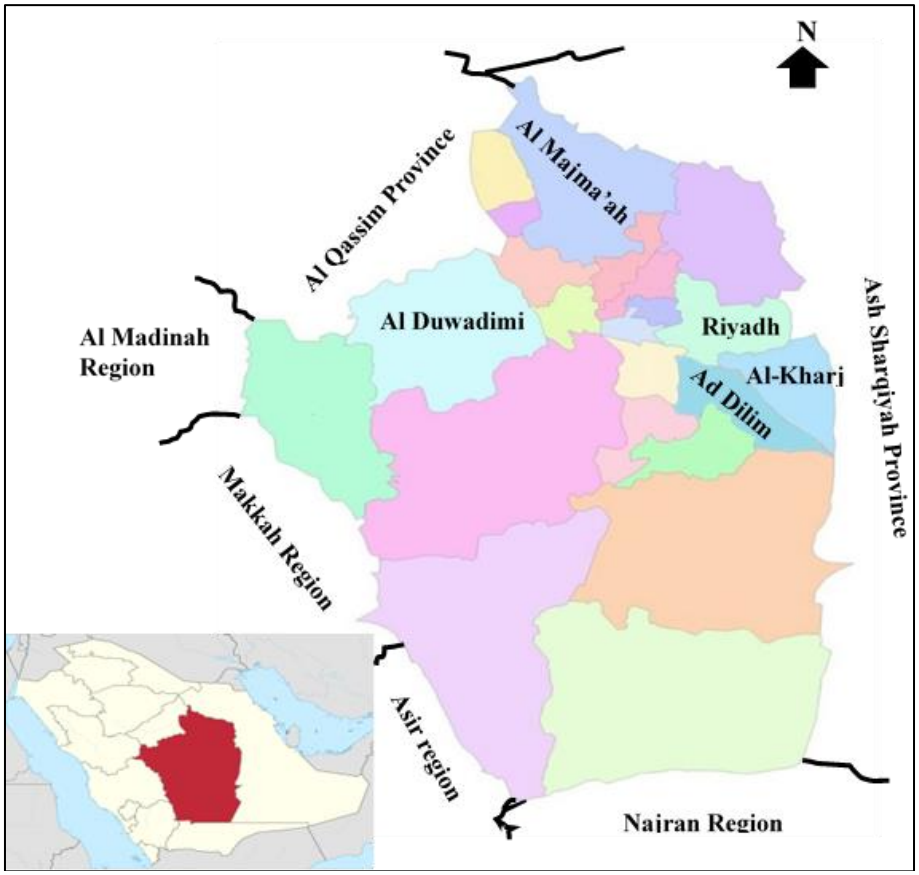

Fig 1: A map of Saudi Arabia showing the location of the surveyed areas (Riyadh city and Governorates of Al Kharj, Al Majma'ah, and Dawadmi) in the Riyadh Province (https://www.wikiwand.com/en/Provinces_of_Saudi_Arabia)

2.3.2. Larval sampling

The larval surveys were conducted during 2017 in 17 areas in Riyadh city and the governorates of Al-Kharj and Ad Dalim in the Riyadh Province. The larvae were collected from potential habitats by dipping method using a standard 350-ml dipper. In each area, the larval survey was done from 08:00 am to 11: 00 am In each habitat, the presence of mosquito larvae was checked by applying at least 20 dips. When allocated, larvae were then sampled by skimming the surface of the water along with the habitat. The collected larvae were kept in well-labeled plastic bottles (500-ml) containing water from the larval habitat. The bottles containing larvae were then placed upright in an ice-box and transported to the Laboratory of the Directorate of Vector-Borne and Zoonotic Diseases at the Ministry of Health in Riyadh city. In the laboratory, the mosquito larvae of the different genus (e.g. *Anopheles*, *Culex*, and *Aedes*). The 4th larval instars were immediately identified to species based on morphological characters. The young stags and (1st and 2nd) and 3rd instars were kept in rearing trays (500 ml), provided with larval food until developed to the 4th larval instar. The 4th larval instars were then examined under dissecting microscope and identified to species level. Moreover, some of the larvae were reared to adults for identification.

2.4. Morphological identification of mosquitoes

The field-collected mosquitoes as adult and larvae (i.e. 4th instars) and the laboratory-reared 1st, 2nd, and 3rd larval instars to 4th instars were examined under the dissecting microscope and identified to species level based on differences in some morphological characters. The adult mosquitoes were identified based on differences in morphological characters of mouthparts (e.g. palps), wings, legs, and abdomen [24, 25]. The morphological characters used for the identification of adult *Anopheles* mosquitoes were the pale and spotted areas in the wing (i.e. the number of pale areas), specking in the legs, banding pattern in the palpi, white spot at the tibiotarsal joint, and/or banding on tarsomeres at the hind legs and the tufted top black scale on each of abdominal segments. The main morphological characters used to differentiate between *Culex* species were the presence or absence of the following features; bands on the proboscis, tarsi, and abdominal terga as well as the white spots on wings. Besides, adult *Aedes* mosquitoes were identified as species based on the numbers

and the position of white lines or spots on the mesonotum of the thorax, the size, and the numbers of white bands on the hind legs.

Identification of mosquito larvae was done using 4th instars. For identification of larvae, morphological characters used were the presence or absence of the siphon, the length of the siphon compared to segment X, and the setae on the siphon (i.e. their shape, length, type, number, or density and their positions, etc…) [26].

2.5. Statistical analysis

The data obtained from this study were analyzed using descriptive analysis.

3. Results

3.1. Mosquito fauna

A total of 2839 specimens of mosquitoes were collected from surveyed areas. The field-collected specimens comprised five mosquito species. These species were *Anopheles dthali*, *An. pretoriensis*, *Culex* (*Cx.*) *pipiens*, *Cx.* (*Cx.*) *quinquefasciatus* and *Aedes aegypti*. All these species were recorded in larval collections; whereas, only *Ae. aegypti*, *Cx. pipiens*, and *Cx. quinquefasciatus* were observed in the adult collection.

3.2. Abundance and sex ratio of mosquitoes

Out of 2839 mosquitoes collected, 2566 were larvae and 273 were adults (Table 2). As shown in table 2, *Cx. quinquefasciatus* was found to be the most abundant species in the area. Besides, *Cx. quinquefasciatus* represented more than 80% of the specimens in the larval collection (Figure 2). However, *Ae. aegypti* formed the bulk of the adult catches 82.0%) was recorded in a higher proportion than *Cx. pipiens* (16; 18.0%) (Figure 3).

Table 2: Numbers of mosquito species collected as adults and larvae in the Riyadh Province from 2012 to 2017.

Species	Numbers of mosquitoes		Total
	Adult	Larvae	
Anopheles dthali	0	24	24
An. pretoriensis	0	3	3
Culex pipiens	16	251	267
Cx. quinquefasciatus	73	2223	2296
Aedes aegypti	184	65	249
Total	273	2566	2839

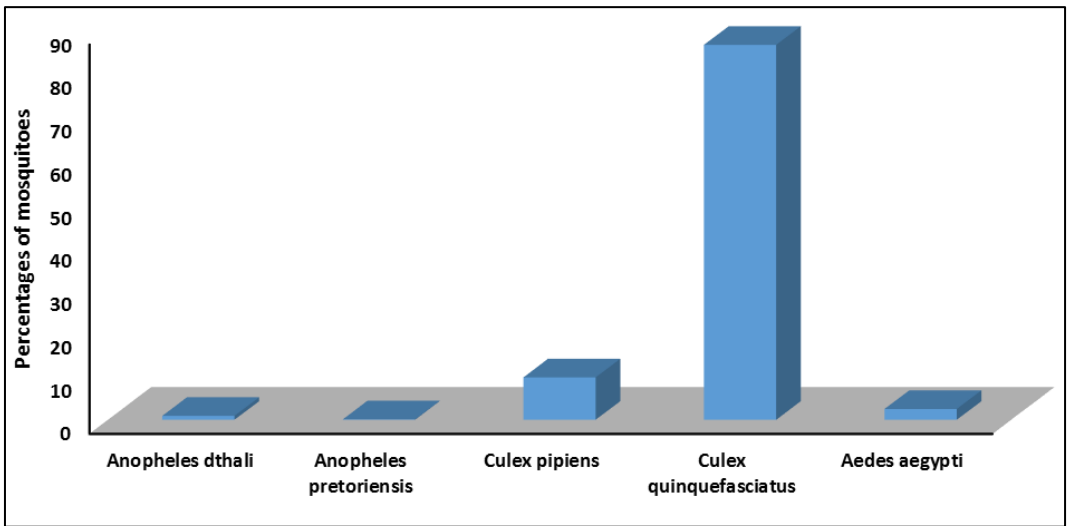

Fig 2: Percentages of mosquito species recorded in the larval collection in the Riyadh Province during 2017.

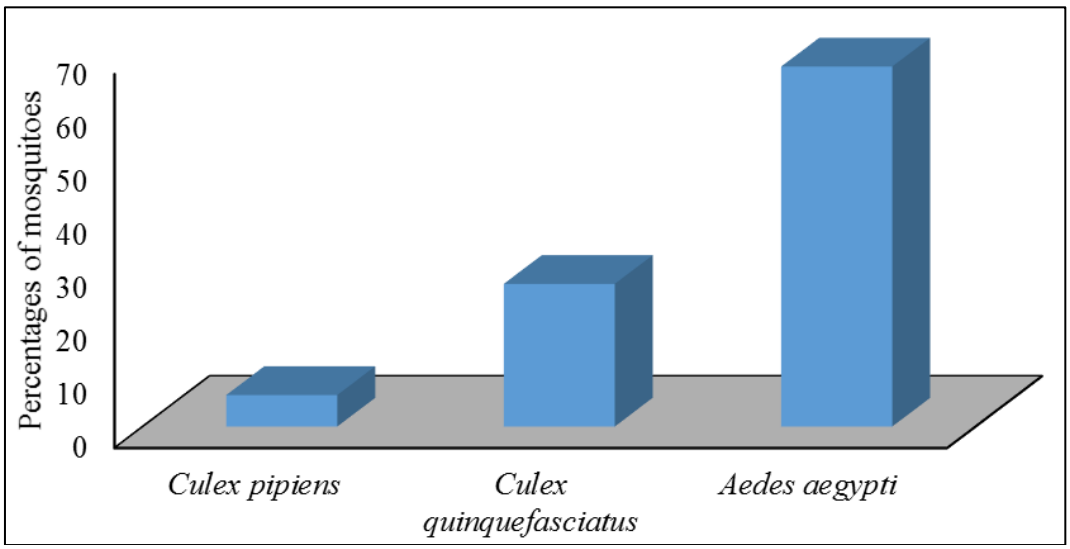

Fig 3: Percentages of mosquito species recorded in the adult collection in the Riyadh Province from 2012 to 2017.

The abundance of mosquitoes in the study was relatively varied between the years. However, *Anopheles* and *Culex* species were collected only during the year 2017. In contrast, and *Ae. aegypti* was collected in different numbers from 2012 – 2017 (Table 3 and figure 3). As shown in table 3 and figure 3, *Ae. aegypti* was recorded in higher numbers in 2013 (80; 32,1%) and 2017 (129; 51,8%).

Table 3: Number of *Aedes aegypti* collected in different surveyed areas in the Riyadh Province from 2012 to 2017.

Governorate/ City	Year	Number of *Aedes aegypti*		Total
		Larvae	Adults	
Riyadh city	2012	0	0	0
Al-Kharj Governorate		0	4	4
Al Majma'ah Governorate		0	10	10
Al Duwadimi Governorate		0	4	4
Riyadh city	2013	0	0	0
Al-Kharj Governorate		0	25	25
Al Majma'ah Governorate		0	37	37
Al Duwadimi Governorate		0	18	18
Riyadh city	2104	0	4	4
Al-Kharj Governorate		0	0	0
Al Majma'ah Governorate		0	10	10
Al Duwadimi Governorate		0	0	0
Riyadh city	2015	0	1	1
sAl-Kharj Governorate		0	1	1
Al Majma'ah Governorate		0	0	0
Al Duwadimi Governorate		0	0	0
Riyadh city	2016	0	0	0
Al-Kharj Governorate		0	3	3
Al Majma'ah Governorate		0	3	3
Al Duwadimi Governorate		0	0	0
Riyadh city	2017	53	13	66
Al-Kharj Governorate		12	0	12
Al Majma'ah Governorate		0	51	51
Al Duwadimi Governorate		0	0	0
Total		65	184	249

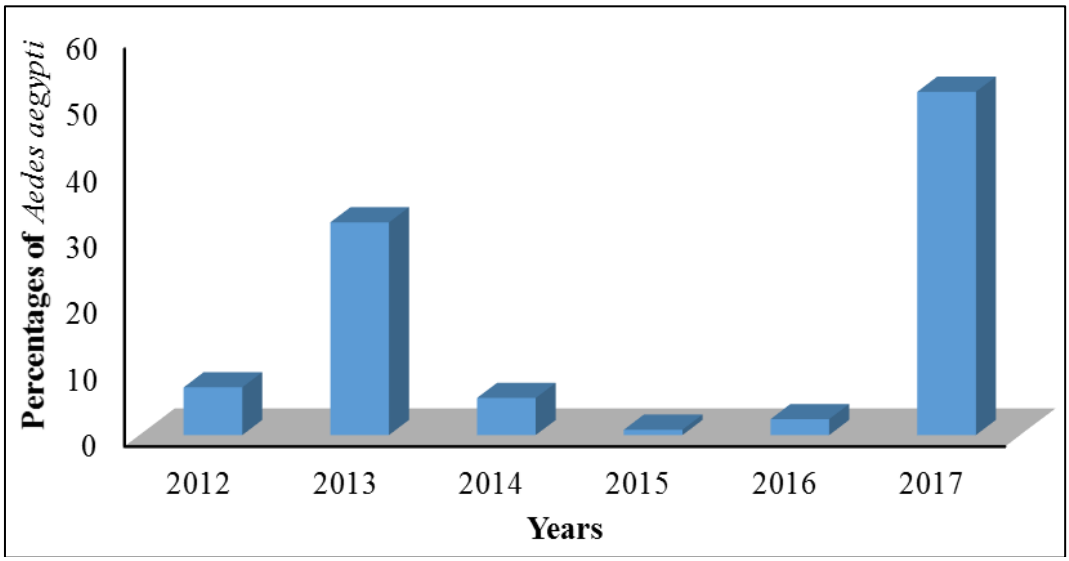

Fig. 4: Percentages of mosquito species recorded in the adult collection in the Riyadh Province from 2012 to 2017.

Table 4 shows the numbers of females and males of mosquitoes and the sex ratio for adult mosquitoes collected in the Riyadh Province. Overall, females were recorded in a higher proportion than males (2.1:1) in adult catches. As shown in the table, females of all species were recorded in high proportions than males.

Table 4: Numbers of males and females and the sex ratio of field-adult collected mosquitoes from the Riyadh Province from 2012 to 2017.

Species	Number of mosquitoes		Total	Sex ratio (Male/Female)
	Males	Females		
Culex pipiens	6	10	16	0.6:1
Cx. quinquefasciatus	29	44	73	0.7:1
Aedes aegypti	30	154	184	0.2:1
Overall	65	208	273	0.3:1

3.3. Distribution of mosquito species in the Riyadh Province

The distributions of mosquito species in the Riyadh Province are depicted in figures 5 and 6. *Anopheles* species were recorded during 2017 only in the larval collection in Riyadh city, and the governorates of Al-Kharj and Ad Dilam (Figure 5). *Culex* mosquitoes were also collected as adults and larvae during 2017 in Riyadh city, and the governorates of Al-Kharj and Ad Dilam (Figure 5). In contrast, *Ae. aegypti* was collected in the Riyadh city, and the governorates of Al-Kharj, Al Duwadimi, and Al Majma'ah as adults (Figure 6) and as larvae in Riyadh city and Al-Kharj governorate.

As shown in table 3, *Ae. aegypti* was recorded as adults in most of the surveyed areas during different years of the study; whereas, the larvae were collected only in 2017 in Riyadh city and Al-Kharj governorate. However, *Ae. aegypti* was absent in the adult catches in the governorates of Al-Kharj and Al Majma'ah during 2014 and 2015, respectively. In Al-Kharj governorate, this species was collected as larvae in 2017. Also, *Ae. aegypti* was recorded in the adult catches in Al Duwadimi Governorate during the years 2012, 2013, and 2017. In Riyadh city, *Ae. aegypti* was recorded in the adult catches in 2014, 2015, and 2017 in different areas in Riyadh city namely; Al Malaz, Umm Al Hamam, and Al dar Albaida areas, respectively. Also, *Ae. aegypti* was recorded in the larval collection in Irqa area in 2017 in Riyadh city.

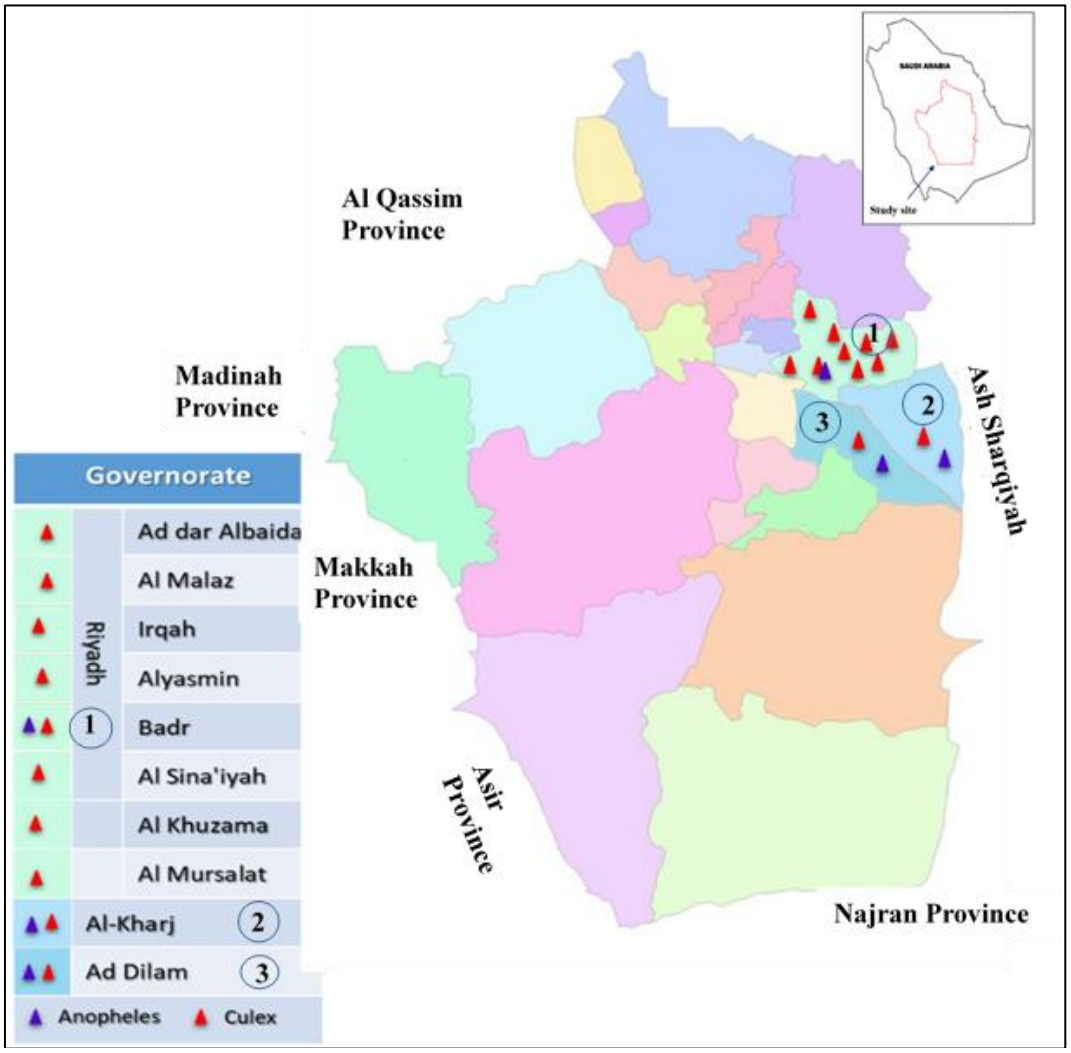

Fig. 5: A map showing the distribution of *Anopheles* and *Culex* mosquitoes in the Riyadh Province.

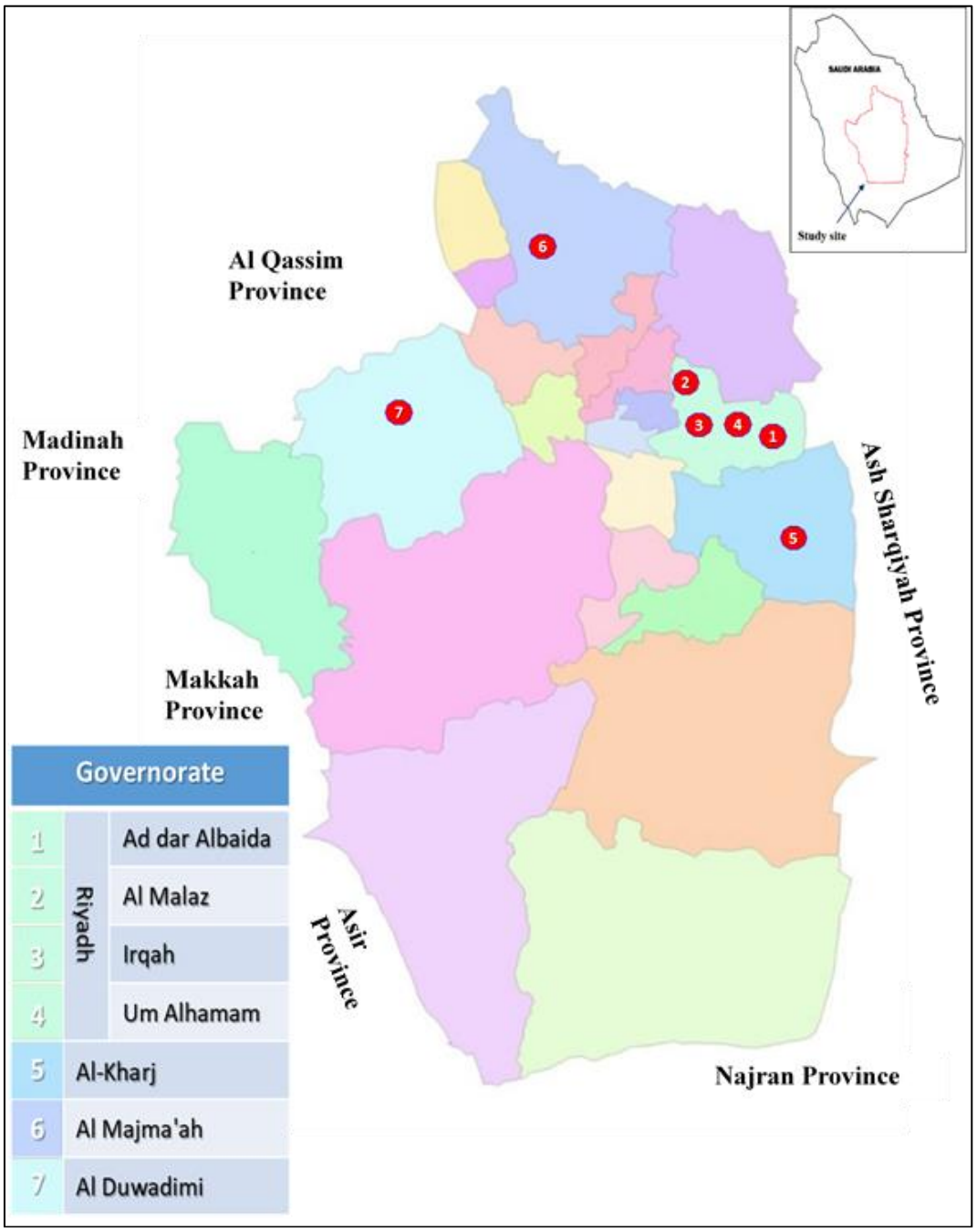

Fig 6: A map showing the distribution of *Aedes aegypti* in the Riyadh Province.

4. Discussion

An entomological survey was conducted in specific places in the Riyadh Province from 2012 to 2017, to identify and determine the occurrence and distribution of the mosquito species. Five mosquito species; *Anopheles dthali*, *An. pretoriensis*, *Culex pipiens*, *Cx. quinquefasciatus* and *Aedes aegypti* were recorded in this The results obtained on mosquito fauna in this study are consistent with findings of previous reports on the general distribution maps of mosquitoes of Saudi Arabia [11]. Despite of *Ae. aegypti*, these species were also recorded in the Riyadh Province [22]. The authors recorded 5 *Anopheles* species and 10 *Culex* mosquitoes in the area. Therefore, this is considered to be the first record of *Ae. aegypti* in the Riyadh Province.

The anopheline mosquitoes in the Riyadh Province comprised 2 species; *viz*. *An. dthali*, *An. pretoriensis*. *Anopheles* (*Cellia*) *dthali* (Patton, 1905), *An.* (*Cellia*) *pretoriensis* (Theobald, 1903). *Anopheles dthali* was recorded in different regions in KSA including the Riyadh region [21, 22, 26, 27]; whereas, *An. pretoriensis* was reported in the Jazan, Riyadh, and Eastern regions of KSA [17, 20, 21]. Both species have never been implicated in the transmission of malaria and/or other mosquito-borne diseases in the country. However, more recently *An. dthali* has been incriminated as a secondary vector of malaria in Jazan Province in the southern region of the country [27]. Besides, *An. dthali* is considered one of the important malaria vectors in Iran [28].

The *Culex* mosquitoes in the study area also included two species; *Cx. pipiens* (the northern house mosquito), and *Cx. quinquefasciatus* (the southern house mosquito). *Culex* (*Cx.*) *pipiens* (Linnaeus, 1758) and *Cx.* (*Cx.*) *quinquefasciatus* (Say, 1823) are considered cosmopolitan species. These species are known vectors of some viral and parasitic diseases in different regions around the world [29- 31]. Similarly, *Cx. pipiens* and

Cx. quinquefasciatus were incriminated as the main vectors of West Nile virus (WNV), and bancroftian filariasis in the KSA [11, 13, 32]. *Culex pipiens* and *Cx. quinquefasciatus* are the most common and predominant mosquito species in different regions of KSA [11, 33, 34]. Also, both species were collected as adults and larval stages from most of the surveyed areas. Hence, the predominance of *Cx. pipiens* and *Cx. quinquefasciatus* in the region might be a risk of transmission of WNV, and Bancroftian filariasis in the future.

Mosquito specimens collected in this study were identified based on morphological characters of adults and larvae (i.e. 4th larval instars) as mentioned before. Out of the five identified species, the invasive mosquito species *Ae. aegypti* is the first record in the Riyadh Province. *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762), the yellow fever mosquito is one of the most common and widely distributed invasive mosquito species worldwide [23, 35]. Currently, it is considered as one of the major vectors of several arboviral infections like yellow fever, dengue fever (DF), chikungunya, and, Zika viruses [36]. *Aedes aegypti* is one of the most common mosquito vectors in KSA especially in the western and southern regions [16, 20, 37, 38]. It represents a serious public health problem in these regions especially Jeddah, Makkah [39], and recently Al Madinah Al Munoarah, and Jazan [10, 37] since it is implicated in the transmission of DF [16].

Aedes aegypti was detected in four out of five surveyed governorates in the Riyadh Province (see figure 5). It was recorded for the first time as adult mosquitoes in 2012 in the governorates of the Al-Kharj (n = 4) and Al Majma'ah (n = 10). The governorates of Al-Kharj and Al Majma'ah are bordered by Ash Sharqiyah Province and Al Qassim Province, respectively. *Aedes aegypti* has never been recorded in Ash Sharqiyah Province (Eastern Province), and Al Qassim Province [17, 40]. Therefore, the finding might indicate that this mosquito species was first introduced to the Al Majma'ah Governorate from *Ae. aegypti* infested regions such as Makkah and Madinah regions that neighboring the Riyadh Province.

During the surveys conducted in 2013, *Ae. aegypti* was collected also as adults in the same governorates but in higher density than before (see table 3). Besides, it was also recorded in a new place which is the Al Duwadimi Governorate (n = 37). Al Duwadimi Governorate is located in the western region of the Provine which is also bordered by Al Qassim Province; however, it is close to Madinah and Makkah regions which are highly infested with *Ae. aegypti.* Therefore, *Ae.aegypti* most probably spread to Al Duwadimi Governorate these regions (i.e. Makkah and Madinah regions). Then after, *Ae. aegypti* was recorded as adults in 2014, 2015, and 2017 from three different areas in Riyadh city. Moreover, *Ae. aegypti* was then recorded as larvae in 2017 in Riyadh city and Al-Kharj Governorate. This species most probably introduced to the province from the neighboring provinces including Makkah, Madinah, Al Qassim, Asir, and Jazan where most of these regions are highly occupied by *Ae. aegypti.* The introduction of *Ae.aegypti* to the province might be through human movement and transportation of different stuff (e.g. water-air conditioners, tires, water containers etc…) from endemic neighboring regions with *Ae. aegypti*. It is known that *Ae, aegypti* spreads to new locations through human transport activities [41].

Dengue fever is endemic in different regions in the KSA with several outbreaks especially in the western region (i.e. Jeddah and Makkah) [4-7]. These endemic regions with DF fever are bordering the Riyadh Province and thus the dengue virus can be exported to the area by infected people either coming from endemic countries or moving to the area from the internal endemic regions and/or introducing infected *Ae.aegypti* through transport activities. During the period 2014 to 2018, approximately about 14 exported cases of DF infections were diagnosed in hospitals and health facilities in Riyadh city [unpublished data]. Therefore, the detection of the vector of DF *Ae. aegypti* in the province is of great concern like in other areas of the world. Besides, the occurrence of this species in the province might be a risk of future transmission of other *Aedes*-borne arboviral diseases like yellow fever, chikungunya virus, and Zika virus which can be introduced to the province due to the movement of the people when they visit other endemic countries with such viruses.

5. Conclusion

This study reports the first record of the *Ae. aegypti* in the Riyadh Province. The study suggests that *Ae. aegypti* might be introduced to the Province from neighboring regions endemic with dengue fever (DF). The occurrence of this mosquito vector might be a risk of transmission of yellow fever, DF, zika virus, and chikungunya virus. Therefore, vector control strategies against both adults and larvae are needed to stop the spread and suppress the densities of the *Ae. aegypti* in the region.

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