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Surveillance of mosquito vectors in Sana'a: Yemen

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Abstract

The mosquito fauna of Yemen is poorly studied and no modern taxonomic keys to mosquitoes of this country are found. This investigation was conducted to provide baseline data about mosquito vectors prevailing in Sana'a, north-west of Yemen. Immature stages of mosquitoes breeding in different aquatic habitats were collected seasonally from nine locations in Sana'a. Results indicated that 2054 mosquito larvae were collected by dipping method during 2015-2016, representing six culicine mosquito species belonging to 3 genera whilst no anopheline mosquito larvae were found. Depending on morphological keys of the fourth instar larvae, such mosquito species were identified as *Culiseta longiareolata* (980 larvae = 47.71%), *Culex pipiens* (559 larvae = 27.22%), *Culex mattinglyi* (252 larvae = 12.27%) *Culex laticinctus* (207 larvae = 10.08%), *Culex (lutzia) tigripes* (34 larvae = 1.67%), and *Aedes aegypti* (22 larvae = 1.07%). Both *Cs. longiareolata* and *Cx. pipiens* were the most predominant species that found in all locations whilst both *Ae. aegypti* and *Cx. (lutzia) tigripes* were found in only one location, Sana'a city and Al-Haymah Ad Dakhiliyah respectively. Out of the six mosquito species, five (*Cs. longiareolata*, *Cx. pipiens*, *Culex laticinctus*, *Culex mattinglyi* and *Ae. aegypti*) are important vectors of diseases and 2 (*Cx. (lutzia) tigripes* and *Cs. longiareolata*) are predaceous in their larval stages. Additionally, both the dengue vector, *Ae. aegypti*, and the predaceous mosquito, *Cx. (lutzia) tigripes*, were detected for the first time in Sana'a city.

Present findings highlighting the importance of regular mosquito surveillance in recording new species as well as identifying mosquito vectors and their preferable breeding sites. Hence, a satisfactory mosquito control program can be raised. Future extensive surveys, studies of cytogenetic and molecular analysis in addition to predation potential of predaceous mosquito larvae are recommended.

Keywords: surveillance, mosquitoes, larvae, vector, sana'a-yemen

1. Introduction

Mosquitoes are related to order Diptera and suborder Nematocera, family Culicidae and three subfamilies, Culicinae and Anophelinae and Toxorhynchitinae. Both Anophelinae and Culicinae subfamilies are cosmopolitan haematophagous insects of medical importance since both have many mosquito vectors^[1, 2]. But the third subfamily has no any medical important. These vectors dramatically increasing with the rapid growth of human populations^[3]. Hence, vector ecology research and larval surveillance are very important requirements for controlling mosquitoes^[4], particularly when prevention is entirely depending on vector control such as Arboviruses^[3].

Yemen is located in the continent of Asia at the south-western edge of the Arabian Peninsula between 12° and 20° latitudes and 41° and 54° longitudes. It is bounded on the north by Saudi Arabia, Oman on the east, the Gulf of Aden on the south and the Red Sea on the west. A total area of Yemen is 527,970 square kilometers and the total population is almost 27,584,213. Geographically it could be divided into four main regions namely; the coastal plains in the west, the western highlands, the eastern highlands, and the Rub al Khali in the east. The climate ranges from semi-humid to semi-arid. Rainy seasons occur during the spring and the summer averages 130 millimeters annually. The flora of Yemen is a mixture of the tropical African, Sudanian plant geographical region and the Saharo-Arabian region.

For the past 5 decades, the environment in Yemen was changed, due to the agricultural activities and the construction of dams and irrigation canals, led to modification in mosquitoes habitat and affected the mosquito fauna. Muturi *et al.*, (2008)^[5] reported that many mosquito vectors remain poorly understood in Southeast Asia.

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Literatures revealed that mosquito fauna of Yemen is poorly studied, and there are no modern taxonomic keys to the species of the major genera found in this country. Unfortunately, there are few scientific studies aimed to increase the knowledge about mosquito identification and distribution in Yemen and most of them if not all, very old and centered on malaria vectors [6-15]. From this point of view, it is necessarily to conduct intensive surveillance and biodiversity studies on mosquitoes in Yemen. Accordingly, the present study was conducted to identify mosquito species prevail in Sana'a (the western highlands of northern Region), Yemen to provide a preliminary information about mosquito vectors and their abundance in this geographic area.

2. Materials and Methods

2.1 Study sites

The present study was carried out in Sana'a, it is located at the middle of the western part of Yemen at 15.36 Northern latitude 44.19 Eastern longitude. However, the Sana'a city is not part of the governorate but instead forms the separate governorate of Amanat Al-Asimah (Fig. 1). The governorate covers an area of 11,864 km². It is a mix of mountainous and valleys areas and has no access to the sea. Agriculture activities and cultivated land are the most important activities in this governorate. The climate is characterized by intense heat and seasonal heavy rains, the average annual temperature is 18 °C and the average temperature in summer is 22 °C and the average temperature in winter is 12 °C in Sana'a Governorate. A total of fifty one sites namely; (Amanat Al-Asimah (3 sites), Haymah Ad Dakhiliah (10 sites), Hamdan (2 sites), Khwlan (6 sites), Bani matar (4 sites), Arhab (1 site) Manakhah (7 sites), Sanhan (16 sites) and Bani Hushaysh (2 site), were selected for mosquito collection. Figure (1) showing coordination of such localities.

2.2 Immature stages collection

The aquatic immature stages (larvae and any pupae) were collected seasonally during 2015-2016 from all selected sites of Sana'a (Figures 1 and 2). Larvae and pupae were sampled using a standard mosquito larval dipper from different breeding sites. These breeding sites included ponds, puddles, construction pits, man mad holes, soaking pits, wells, swamps, irrigation ditches, marshes and rocky pools (Figure 2). Samples were placed into plastic container half filled with water from the sampling site, then they were taken to the Entomology laboratory: Biological Science Department, Faculty of Science, Sana'a University. Sample of each breeding site was divided into two halves. The first one was preserved in 70% ethanol inside glass vials whilst the second half was left alive and reared inside mosquito breeders (Bio quip) till the emergence into adults.

2.3 Morphological Identification

Collected samples were identified based on the morphological characteristics of the 4th larval instar using standard identification keys of larvae [2, 16-20]. Identified specimens were preserved as a reference collection in the Medical Entomology laboratory, Biological Sciences Department, Faculty of Science, Sana'a University.

2.4 Statistical analysis

Variation in mosquito larvae densities in different seasons was analyzed using SAS v9.

3. Results and Discussion

Fifty one various sites were surveyed as potential aquatic mosquito larvae breeding habitats in 9 locations of Sana'a, Yemen (Figure 1) but 41 sites of them (80 %) were found positive. A total of 2054 of mosquito larvae were collected representing 6 mosquito species from family of Culicidae, order Diptera. Results indicated that one aedine mosquito species "*Ae. aegypti*" and 5 culicine mosquito species "*Cx. laticinctus*, *Cx. mattinglyi*, *Cx. pipiens*, *Cx. (lutzia) tigripes* and *Cs. longiareolata*" were encountered during the present survey in Sana'a, Yemen (Table 1 and 2) during 2015-2016. The aedine mosquito species "*Ae. aegypti*" was encountered in one location (Amanat Al-Asimah, Sana'a city) as well as *Cx. (lutzia) tigripes* that was encountered in Al Haymah Ad Dakhiliah, Sana'a region whilst both *Cx. pipiens* and *Cs. longiareolata* were encountered in the all 9 locations in Sana'a. both *Cx. laticinctus* and *Cx. mattinglyi* were collected from all locations except for Amanat Al-Asimah. Larvae of both *Cx. (lutzia) tigripes* and *Cs. longiareolata* are an important agent for biological mosquitos' control whilst the other three species are vectors of diseases transmitted to humans.

Out of 2054 larvae collected from the monitored locations, 980 *Cs. longiareolata* larvae (representing 47.71%), 559 *Cx. pipiens* larvae (representing 27.22%), *Cx. mattinglyi* larvae (representing 12.27%), *Cx. laticinctus* larvae (representing 10.08%), 34 *Cx. (lut) tigripes* larvae (representing 1.67 %), and 22 *Ae. aegypti* larvae (representing 1.07%) were identified (Table 1). Results also clearly indicated that *Cs. longiareolata* was not only the most common but also the most dominant and abundant genus and species in the same time in this study, followed by *Cx. pipiens*, *Cx. mattinglyi*, *Cx. laticinctus*, *Cx. (Lutzia) tigripes* and *Ae. aegypti*. According to breeding habitat, *Cx. pipiens* larvae were found in a wide range of habitats in all locations but with different densities, *Cx. (Lutzia) tigripes* larvae were found in rocky pools and *Ae. aegypti* larvae were collected from standing water near the campus of Sana'a University. *Cs. longiareolata* larvae were found in association with *Cx. laticinctus*, *Cx. mattinglyi* *Cx. (Lutzia) tigripes* and *Cx. pipiens* larvae in the same breeding sites in the present study. Furthermore, it is worth to mention that this study revealed that this is the first time to record *Ae. aegypti* in Sana'a city.

Cs. longiareolata bred in fresh, clean water sources such as irrigation ditches, water reservoirs and occurs in southern Palearctic region from North Africa to India [21] Wood *et al.*, [22] (1979) mentioned that it is often most abundant near seasonally flooded river bottomland forests and often breeds in shallow grass filled depressions in pastures or in temporary woodland pools. Although adults of this species are known as vectors of diseases such as West Nile encephalitis, brucellosis, avian influenza [23], the larvae are known as mosquito larvae predators [24]. These results agree with similar studies conducted in Iran [25-28]. Statistical analysis (Table 2) showed that this mosquito was statistically more abundant in summer season than other seasons (F=71.84, df =3, P < 0.05). Identification of *Cx. pipiens* complex related species is still highly controversial [29, 30]. Results of the present survey demonstrated that *Cx. pipiens* was found to be the second most common and abundant mosquito species after *Cs. longiareolata*. They were collected from all selected areas either in Sana'a city or in Sana'a governorates. These results agree with Harbach (1988) [18] who reported that *Cx. pipiens*

was very widely distributed in the eastern areas of the Afro tropical Region such as Yemen and the southwestern part of the Saudi Arabia. Similar studies reported this species as the most prevalent among surveyed mosquitoes [29, 31-33]. This mosquito has a close association with man whereas its larvae inhabit artificial, water reservoirs especially those polluted with sewerage or barnyard wastes [22]. This species was found sharing the same habitats with *Cs. longiareolata* and *Cx. (Lutzia) tigripes* larvae in the present survey. Data in table (2) showed that *Cx pipiens* is statistically more abundant in autumn compared to the other seasons ($F= 21.56$, $df=3$, $P < 0.05$).

Cx mattinglyi was the third common mosquito species collected in the present study. It is known only from the highlands of the Yemen and a single locality near its northern border in Saudi Arabia, southwestern mountains of the Arabian Peninsula where it is restricted to higher terrain [18]. On other hand, Knight (1953) [6] found the immature stages of this species in a cement watering trough, a broad open well, a large cement basin, and a large cement tank. Statistically (table 2), this mosquito was more abundant in summer ($F= 11.54$, $df= 3$, $P < 0.05$).

Cx laticinctus was the fourth common mosquito species. It is sympatric throughout the arid lands of northern Africa and southwestern Asia and Mediterranean regions [17] and it was recorded in Arab countries including Yemen [17, 18]. It seems to have been more common in the past than it is today. This mosquito usually occurs in stream pools, rock pools, swamps, springs, irrigation ditches, fresh water, slightly brackish water, artificial containers (cisterns, tanks, barrels, wells, concrete basins, and similar structures) and temporary ground water. Like the previously mentioned three mosquito species, *Cx laticinctus* was statistically (table 2) more abundant in summer ($F= 258.92$, $df= 3$, $P < 0.05$).

Lutzia might be a very ancient derivative of *Culex* but it was described as independent genus [34]. Several species related to this genus were identified from Central and South America, Asia, Australasia and Africa [34]. The low number of *Cx. (Lutzia) tigripes* larvae (34) collected in the present survey were from one location, Al- Haymah Ad Dakhiliyah (Figure 1 and Table 1). Although this species has been reported as unimportant in diseases transmission, *Lutzia* mosquito's in particular larval stage showed predation capacity against other mosquito vectors [35, 36, 37]. Statistical analysis (Table 2) showed that this mosquito was statistically more abundant in summer and spring seasons than other seasons ($F=4.20$, $df=3$, $P < 0.05$).

The present study revealed for the first time the presence of *Ae. aegypti* in Sana city. Literatures indicated that no early mosquito survey before this study recorded any stages of *Ae. aegypti* in Sana'a city. Larvae were collected from breeding site and found in restricted very close to Sana'a University in Sana'a city. This species was the most important dengue fever vector in Yemen. Recently several areas experienced dengue outbreaks and the disease became endemic in a number of

governorates over the last 10 years [38, 39]. Additionally, it is well known as primary vector of other viral diseases including yellow fever and chikungunya. It is an important mosquito species and can be found in the different man-made containers, storage of water in cement tanks, plastic containers, cans and tires [40]. It is often most abundant near seasonally flooded. Accordingly, occurrence of *Ae. aegypti* in Sana'a city is of great concern from the medical point of view. The introduction of this species to Sana'a city could be via either used tires or vehicles traveling from infested areas such as Taiz, Hodaidah, Aden and Hadramout. If this mosquito adapted to the environmental conditions in Sana'a city, it will be considered as threat and people who live in Sana'a city will be at risk. The lower number (22) of collected larvae indicates that this species is at the low level of density, but the population of this species can grow and spread sooner. Once *Ae. aegypti* or any species of mosquitoes is established in an area, it is difficult to control or eradicate and constant surveillance and appropriate control strategies are required.

According to the seasonal prevalence, the present survey revealed that mosquitoes are present in Sana'a throughout the year with significant increase in the density during summer and spring then dramatically decreased during autumn and winter seasons (Figure 3). Such change in densities could be attributed to many factors such as air and water temperatures, rainfall and human activities particularly agricultural practices. Present results are consistent with other investigations [15, 41-47] that indicated that meteorological parameters are very important factors affecting the spatial and temporal distribution of mosquito vectors as well as human activities which play an important role in the availability of host for blood meal leading to contribute the distribution and abundance of the mosquito vectors.

Unlike culicine mosquitoes, no anopheline mosquitoes were encountered in the present study. This could be due to the altitude, rainfall and topography of Sana'a which play the role factors in mosquito's distribution. The high of the mountains of Sana'a reach more than 2000 asl above sea level. It extends from 2150 to 2200 asl. At this level there are no studies in Yemen confirmed the presence of anopheline mosquitoes [48]. Additionally, the number of locations covered during this study was 9 districts out of 28 districts and if more locations were surveyed, anopheline mosquitoes may be encountered.

Although these 6 mosquito species are previously recognized and recorded from Yemen [8]. Literatures indicated huge difference in Yemeni mosquito fauna. For instance, Harten and Wagener [12] listed 48 mosquito species whilst the online taxonomic website of the Walter Reed Biosystematics Unit (WRBU) listed a total of 19 species. Such difference in the number of mosquito species between these data and the present study could be due to the number of location covered during all these surveys. Furthermore, the present study was performed in Sana'a but the other studies were conducted in other regions.

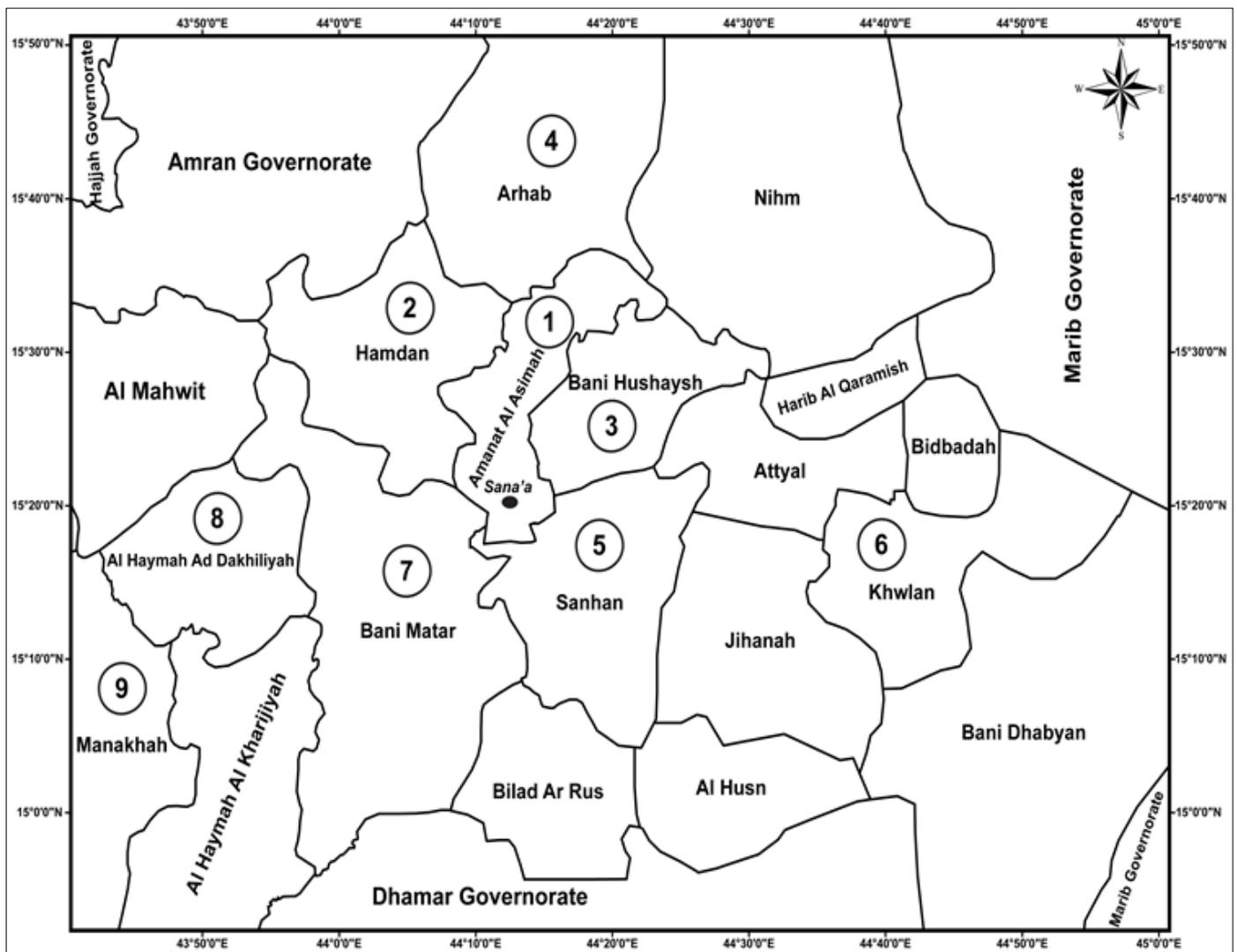
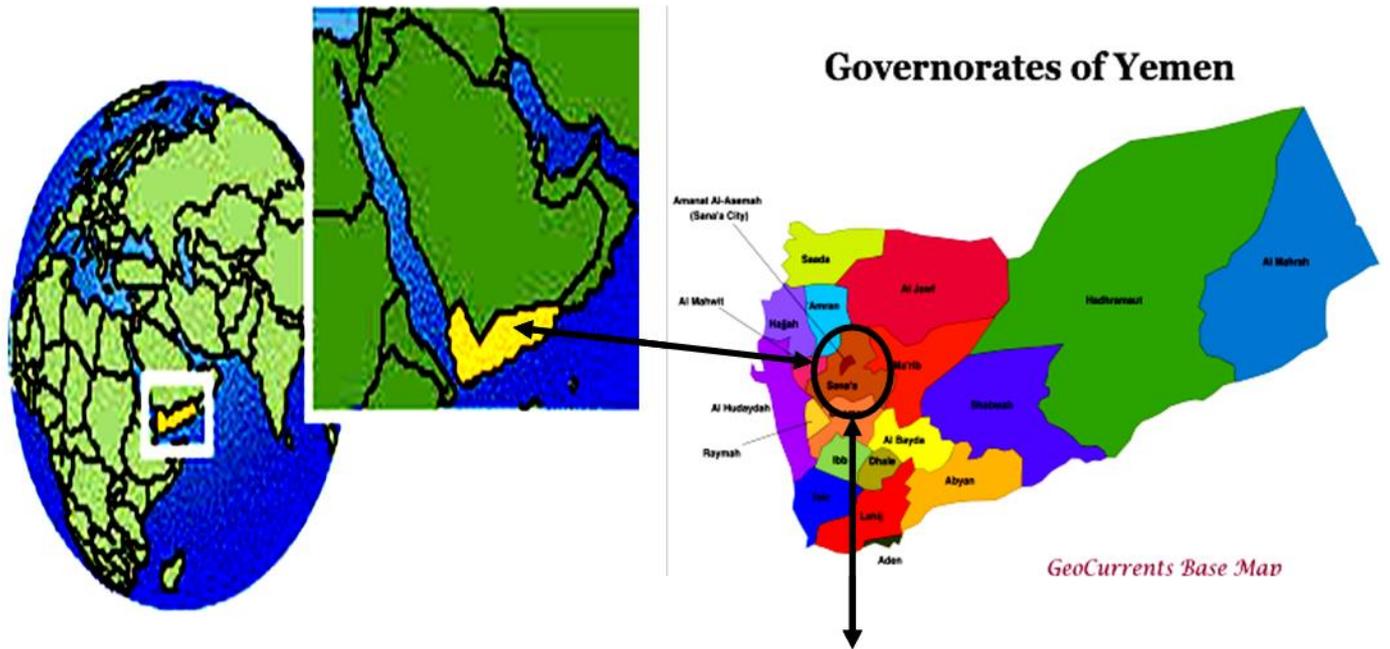


Fig 1: Map showing coordination of studied locations in Sana'a -Yemen



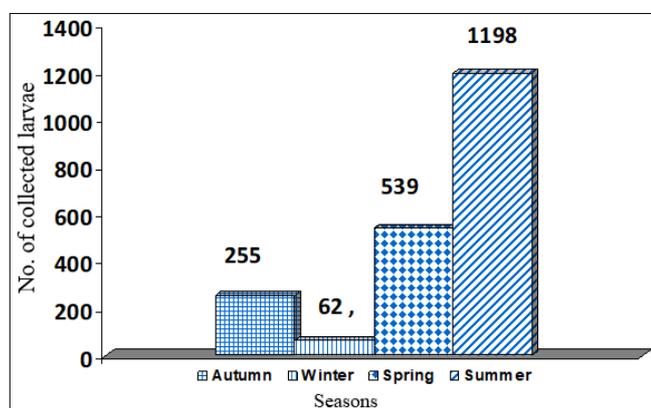
Fig 2: Collecting immature stages of mosquito from the selected sites during 2015-2016 in Sana'a, Yemen.

Table 1: Total number of mosquito species collected from Sana'a during 2015-2016.

No. No	Location	Mosquito species					
		<i>Aedes aegypti</i>	<i>Culex laticinctus</i>	<i>Culex mattinglyi</i>	<i>Culex (Lutzia) tigripes</i>	<i>Culex pipiens</i>	<i>Culiseta longiareolata</i>
1	Amanat Al-Asimah	22	0	0	0	8	83
2	Hamdan	0	5	4	0	24	48
3	Bani Hushaysh	0	10	12	0	66	144
4	Arhab	0	14	20	0	151	220
5	Sanhan	0	9	11	0	21	33
6	Khwlán	0	8	6	0	86	178
7	Bani Matar	0	18	14	0	102	125
8	Al Haymah Ad Dakhiliyah	0	120	170	34	75	112
9	Manakhah	0	22	15	0	26	37
Total		22	207	252	34	559	980

Table 2: Seasonal abundance of mosquito species collected from Sana'a during 2015-2016.

Season	Mosquitoes Species					
	<i>Aedes aegypti</i>	<i>Culex laticinctus</i>	<i>Culex mattinglyi</i>	<i>Culex (Lutzia) tigripes</i>	<i>Culex pipiens</i>	<i>Culiseta longiareolata</i>
Autumn	1 ^c	25 ^c	48 ^{cb}	1 ^b	80 ^{bc}	100 ^c
Winter	0 ^c	5 ^d	4 ^c	2 ^b	11 ^c	40 ^c
Spring	7 ^b	47 ^b	80 ^{ab}	12 ^{ab}	163 ^b	230 ^b
Summer	14 ^a	130 ^a	120 ^a	19 ^a	305 ^a	610 ^a
<i>P</i> value >	0.0016	0.001	0.0177	0.1430	0.0032	0.0001
R-Square	0.94	0.99	0.85	0.68	0.92	0.97

**Fig 3:** Seasonal abundance of mosquito larvae collected during 2015-2016 in Sana'a, Yemen.

4. Conclusion

Six culicid mosquito species belong to three genera were identified by examination of larval morphology and chaetotaxy in the present study. Both *Cs. longiareolata* and *Cx. pipiens* are the prevalent mosquito species in several habitats and breeding sites. Although this survey was mainly for mosquito vectors, a couple of the collected mosquito species are known as predators in the larval stage against other mosquito larvae. Among the outstanding findings of the present work are the recording of the dengue vector from Sana'a city for the first time and the absence of the anopheline mosquitoes. Moreover, it indicated that identification of both types of habitats breeding sites and vector mosquitoes are necessary not only for early estimation of mosquitos' density but also for effective control of arboreal and other diseases transmitted by mosquitoes. In addition to studies on predation potential of predaceous mosquito larvae collected in the present work, more mosquito surveys as well as cytogenetic and molecular studies are recommended for accurate identification of mosquito species, particularly mosquito vectors, spreading in Yemen.

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6. Conflict of interest

Authors declare that, there is no conflict of interest.

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