ENTOMOLOGY

A faunal study on medically important mosquitoes (Diptera: Culicidae) in Qir and Karzin from Fars province, southern Iran, during 2017-18

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Abstract

Mosquitoes have always played an important role in transmitting a wide range of viral and parasitic diseases to humans and animals. Given that so far there has been no study on the fauna, distribution and characteristics of mosquito habitats in Qir and Karzin County, we decided to investigate these unknown data in this important tropical area in south of Iran for the first time. Adult mosquitoes were collected by hand-catch and total catch methods from indoor and outdoor places in Qir and Karzin Counties from four different geographical regions during the activity seasons of mosquitoes in 2017-18. Also, larvae were collected from aquatic habitats using WHO recommended standard dipper. Collected mosquitoes were identified using valid taxonomic keys. A total of 1884 specimens of Culicidae mosquitoes (1103 adults and 781 larvae) were collected from twenty-one sampling areas during the mosquito activity seasons in Qir and Karzin County. Totally 11 species in 3 genera (7 *Culex*, 3 *Anopheles* and 1 *Culiseta* species) were identified. The dominant larva was *Cx. laticinctus* with a total number of 227 (29%), and *Cx. sinaiticus* with 407 specimens (36.9%) which was the most frequent adult mosquito. Most larval habitats in this area were sunny and almost without vegetation breeding sites with temporary, turbid and stagnant water. In this region, compared to the area of the County, there was considerable species diversity for mosquitoes. This phenomenon is very important from an ecological and health point of view. Due to the existence of some important vectors of arthropod-borne diseases in this region, the health system should be alert and continuous and accurate monitoring of these vectors should be included in the routine vector-borne diseases control program.

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Key words: Mosquito, faunal study, aquatic habitats, Qir and Karzin, Iran.

Acknowledgements: Authors are thankful to the assistance of vice-chancellorship for research and technology at Shiraz University of Medical Sciences (SUMS). This paper has partly been derived from, and supported by Ms. Zahra Hoseini Master of Science thesis (Grant No: 1396-01-04-16676) in Medical Entomology, at School of Health, SUMS.

Contributions: ZH and AB designed and performed experiments, analyzed data and co-wrote the paper. ZH, AB, KA, and HA performed experiments and co-wrote the paper. AB, KA, and HA supervised the research.

Availability of data and materials: All data are available.

Conflict of interest: No conflict or competing financial interests exists.

Funding: Not applicable

Received for publication: 1 October 2020.
Revision received: 24 November 2020.
Accepted for publication: 25 November 2020.

Journal of Entomological and Acarological Research 2021; 53:9378
doi:10.4081/jear.2021.9378

Introduction

Mosquitoes have always played an important role in transmitting a wide range of viral and parasitic diseases to humans and animals. More than half of the world's population lives in areas at risk of being bitten and infected by mosquitoes that carry important pathogens such as malaria, dengue fever, chikungunya, West Nile fever, Japanese encephalitis, and filariasis (Keshavarzi *et al*., 2017; Soltani *et al*., 2017).

Although about three-quarters of all mosquito species are distributed in humid, tropical, and subtropical regions, mosquitoes are not the only specific problem in these areas. They also irritate people with their bites and are able to transmit pathogens to humans in the other part of the world (Reiter, 2001).

Mosquitoes are extremely successful creatures because of their ability to adapt to different habitats. They are found all over the world except in polar areas (Reiter, 2001). Mosquito larvae

have the power to colonize and live in a wide range of temporary and permanent waters, highly polluted and clean, stagnant or running, and even the smallest places such as buckets, pots, and used tires, footprints of animals and hole of plants.

According to the updated Iranian checklist, Culicidae family includes two subfamilies Anophelinae and Culicinae, 69 species and 3 subspecies representing seven (*Anopheles* Meigen, 1818, *Uranotaenia* Lynch Arribalzaga, 1891, *Culiseta* Felt, 1904, *Coquillettidia* Dyar, 1905, *Culex* Linnaeus, 1758, *Aedes* Meigen, 1818, and *Ochlerotatus* Reinert, 2000) or 11 genera depending on the generic classification of aedines (Azari-Hamidian *et al*., 2019; Edrissian, 2006). Seven species of the genus *Anopheles* Meigen, 1818, including *Anopheles sacharovi* Favre, *An. culicifacies* Giles *An. maculipennis* Meigen, *An. fluviatilis* James, *An. dthali* Patton, *An. superpictus* Grassi, and *An. stephensi* Liston are main vectors of malaria in Iran. *An. pulcherrimus* is another potential vector of this disease in the southeast of the country (Edrissian, 2006; Keshavarzi *et al*., 2017; S. H. Moosa-Kazemi, Zahirnia, Sharifi, & Davari, 2015).

An. hyrcanus has recently been reported as a possible vector of malaria using PCR technique in Guilan province (Gholizadeh, Zakeri, Djadid, Jazayeri, & Rad, 2009; Keshavarzi *et al*., 2017). West Nile and Sindbis viruses, as well as *Dirofilaria immitis* and *D. repens* (causative agents of Dirofilariasis) transmitted by mosquitoes, have been reported in Iran (Azari-Hamidian, 2011; Kazemi, Karimian, & Davari, 2010). The risk of some mosquito-borne arbovirus diseases such as Japanese encephalitis (JE) and Rift Valley fever is probable in the Mediterranean region, including Iran (Banafshi *et al*., 2013; Moosa-Kazemi *et al*., 2015; Navidpour, Vazirianzadeh, Harbach, Jahanifard, & Moravvej, 2012; S. Nikookar *et al*., 2016; Ramesh *et al*., 2015; Soltani *et al*., 2017).

Scattered studies on mosquito fauna have been conducted in different parts of Fars province. In one of them, 5 different genera and 17 species of mosquitoes were reported from selected areas of the Fars province including *An. dthali*, *An. fluviatilis, An. stephensi, An. superpictus, Culex quinquefasciatus, Cx. mimeticus, Cx. perexiguus, Cx. pipiens, Cx. sinaiticus, Cx. tritaeniorhynchus, Cx. torrentium Cx. bitaeniorhyns, Ochlerotatus caspius, Cx. theileri, Cx. modestus, Culiseta longiareolata* and *Aedes vexans* (Keshavarzi *et al*., 2017).

Also, in another study, 3 genera and 6 species of Culicidae with different frequencies were reported from Firouzabad County in Fars Province, including *An. superpictus, An. d'thali, Cx. sinaiticus, Cx. theileri, Cx. mimeticus*, and *Cu. longiareolata* (Soltani *et al*., 2017).

Qir and Karzin County is located in the tropical region of Iran and due to having suitable climate conditions for the growth and reproduction of mosquitoes, so the possibility of transmission of mosquito-borne diseases in this area is very likely. Given that so far there has been no study on the fauna distribution and characteristics of mosquito habitats in Qir and Karzin County, we decided to investigate these unknown data in this important tropical area in south of Iran for the first time.

Materials and methods

Study area

Fars Province is one of 31 provinces of Iran and is located in the south of Iran (29.62°N, 52.53°E). Qir and Karzin County is located in the southwest of Fars province. It is between N 28°32' to 28°54' latitude and E 52°6' to 53°13' longitude of the Greenwich meridian (N 28.48415°, E 52.99710°) with an area of about 98.3402 km^2. Based on the Köppen climate classification, this county is located in the semi-arid areas of the earth with rainy winter and hot dry summer.The maximum temperature is 46 °C in the summer and the minimum temperature is 25.1°C in the winter. The mean precipitation of Qir and Karzin County is 270 mm per year. Qir and Karzin County has borders with Firuzabad, Jahrom, Khonj and Farashband counties. Sampling was carried out in four regions (including Qir, Efzar, Karzin and Emam Shahr) in the mosquito activity seasons (From April to December) monthly (Figure 1).

Mosquitoes collecting and identifying

Adult mosquitoes were collected by hand-catch and total catch methods from indoor and outdoor places (human and animal) (Bodman & Stewart, 1948; Crump *et al*., 2013; Van Rooyen, Bowie, & Krikorian, 1944) (Figure 2). All needed data like date, location and hour of collecting were recorded along with mosquito species. Adult specimens of mosquitoes were pinned and kept at the Museum of Medical Entomology (Shiraz University of Medical Sciences, Department of Medical Entomology). In an innovative method, wasted tires were placed in the target places and filled with water to attract mosquitoes for resting in these moist environments. Two to three days after using tires, they were covered with nets with small pores. Then they were shaken so that mosquitoes, which were resting, flied upwards and got caught by the net. The aspirator was then inserted slowly from the corner of net and mosquitoes were collected and transported into the cups. The caught mosquitoes were transferred alive to the Medical Entomology Laboratory for further studies. After pinning, the adult mosquitoes were identified using valid taxonomic keys (Shahyad Azari-Hamidian & Harbach, 2009; Shahgudian, 1960).

Mosquito larvae were collected by Dipping Method. In each habitat, using a standard dipper, larvae were collected from ten different points in the mosquito larval habitat (Figure 2).

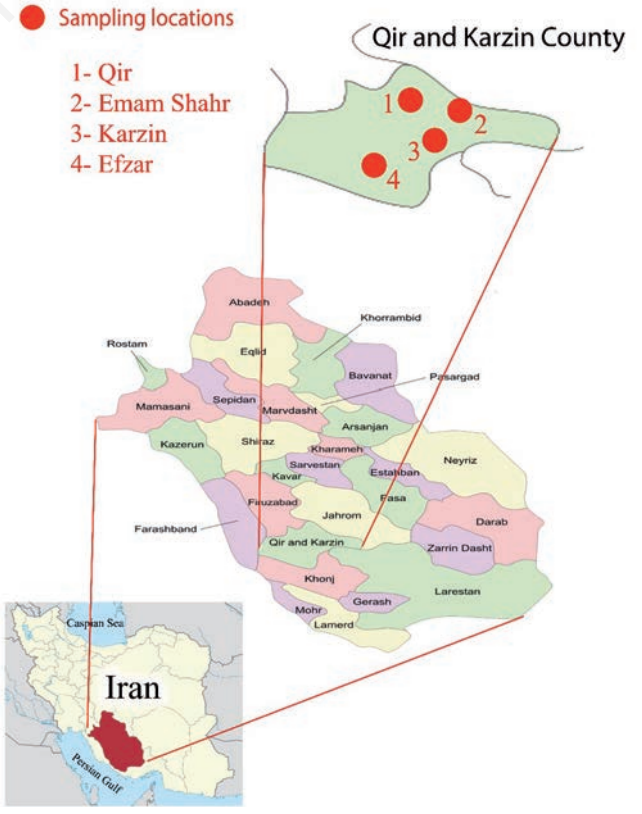

Figure 1. Map of Fars Province, Qir and Karzin County and sampling locations.

Habitats included narrow streams, water collected in pits, animal drinking vessels, rice fields and artificial breeding places. After catching the larvae, they were poured into a bottle and taken to the entomology insectarium. Some of the caught larvae were reared to adult stage and some of them were mounted for identification. Information on the date, place of collecting and type of larval habitat were registered separately. Geographical characteristics (altitude, longitude and latitude) of each sampling area were determined using a GPS device. The collected larvae were transferred to the entomological laboratory in Shiraz health school and they were identified using valid identification keys (Shahyad Azari-Hamidian & Harbach, 2009; Shahgudian, 1960). In this study, *Ochlerotatus* was considered as a separate genus according to Reinert, 2000. The mosquito name abbreviations follow Reinert, 2009.

Results

Mosquito faunal survey

In this study, a total of 1884 larvae and adult specimens of Culicidae mosquitoes were collected and identified through twenty-one sampling operations during spring, summer and autumn from 4 areas of Qir and Karzin County including Qir, Efzar, Karzin and Emam Shahr. Of these, 1103 adult specimens and 781 larval specimens were obtained from all sampling sites. This study showed that a total of 11 species in 3 genera were active in the studied areas. These are 7 species belonging to the genus *Culex*, 3 species belonging to *Anopheles* and 1 species belonging to the genus *Culiseta*. The number and type of identified species of different genera are presented in detail based on their sampling locations and stage of life in Tables 1-3.

Sampling was performed in the warm months of the year, which was coincided with the mosquito activity season. The highest frequency of adults was belonged to the species of *Cx. sinaiticus* with 407 specimens (36.9%) (Table 2). The highest frequency among all collected larvae in the studied areas belonged to *Cx. laticinctus* with a total number of 227 (29%), which put it as the dominant larval species in this area (Table 3).

Larval habitat characteristics of mosquitoes

During the study on the larval breeding places of the collected specimens in the studied areas, it was found that most of the larval habitats were temporary and stagnant. Among the larval habitats, the most collected samples belonged to the species of *Cx. laticinctus*. This species was collected from sunny, semi-shady and vege-

Figure 2. Using of different sampling techniques for collecting of different life stages of mosquitoes in selected sites of Qir and Karzin County, Fars Province during 2017-18. A: Collecting with the adult stage of mosquitoes by total catch method. B: Collecting with the adult stage of mosquitoes using waste tires that filled with water and then covered by a net as a suitable resting/ovipositing place. C: Larval sampling by a standard dipper from a natural breeding habitat. D: Larval sampling from a rice filed using a standard dipper.

tated habitats. Most larval habitats in these areas were sunny and almost without vegetation breeding sites with temporary, turbid and stagnant water. *Cx. torrentium* and *Cx. tritaenorhynchus* were only collected as larvae.

Discussion

Mosquitoes are remarkably adapted to living with humans and domestic animals and are one of the largest vectors of pathogens in the world. Because of their involvement in the transmission of various infectious diseases, mosquitoes are considered a serious threat to public health (AzariI-Hamidian *et al*., 2009). At present, rural areas of Fars province are part of malarial areas in Iran, where the disease is reported sporadically every few years.

Worldwide, Culicidae are classified into 2 subfamilies and 112 genera (Harbach, 2007). There is scattered information about mosquito fauna in Iran (Aaim, Manouchehri, & Ershadi, 1985). Species and environmental characteristics of Culicidae mosquitoes have been reported in Isfahan, Gilan, Kurdistan, Sistan-Baluchistan and Hormozgan provinces (Azari-Hamidian, 2011; Banafshi *et al*., 2013; Moosa-Kazemi, Vatandoost, Nikookar, & Fathian, 2009; Moosa-Kazemi *et al*., 2015). Collecting basic data on population composition, abundance and diversity of mosquito species is essential due to their role as vector of various human and

Table 1. The distribution of the identified mosquitoes in different sampling locations of Qir and Karzin County, Fars Province during 2017-18.

Genus	Species	Different sampling locations			
		Karzin	Emam Shahr	Efzar	Qir
Culex	*Cx. pipiens*	*	*	*	*
	Cx. sinaiticus	*	*	*	*
	Cx. bitaeniorhynchus			*	
	Cx. theileri			*	*
	Cx. laticinctus			*	*
	Cx. torrentium	*	*		
	Cx. tritaeniorhynchus	*	*		
Anopheles	*An. stephensi*	*	*	*	*
	An. dthali	*	*	*	*
	An. superpictus			*	
Culiseta	*Cu. longiareolata*	*	*	*	*

Table 2. Number and percentage of mosquito adults separately sorted by sampling areas in Qir and Karzin County, Fars Province.

Species	No. of collected adult mosquitoes in different sampling locations				Total number	Percentage
	Karzin	Emam Shahr	Efzar	Qir		
Cx. pipiens	17	25	26	69	137	12.42
Cx. sinaiticus	30	102	220	55	407	36.9
Cx. bitaeniorhynchus	0	0	3	0	3	0.272
Cx. theileri	0	0	11	5	16	1.451
Cx. laticinctus	0	0	6	3	9	0.816
An. stephensi	33	41	28	22	124	11.24
An. dthali	15	29	52	58	154	13.96
An. superpictus	0	3	3	0	6	0.544
Cu. longiareolata	38	31	55	123	247	22.39
Total	133	231	404	335	1103	100

Table 3. Number and percentage of mosquito larvae separately sorted by sampling areas in Qir and Karzin County, Fars Province.

Species	No. of collected larvae mosquitoes in different sampling locations				Total number	Percentage
	Karzin	Emam Shahr	Efzar	Qir		
Cx. pipiens	38	40	52	20	150	19.21
Cx. laticinctus	58	84	50	35	227	29.07
Cx. torrentium	4	28	0	0	32	4.097
Cx. tritaeniorhynchus	3	17	0	0	20	2.561
An. stephensi	27	30	50	35	142	18.18
An. dthali	52	48	47	63	210	26.89
Total	182	247	199	153	781	100

animal diseases (Keshavarzi *et al*., 2017; Marini *et al*., 2016).

In this study, a total of 11 species in 3 genera were identified based on the morphological traits of larvae and adults. Of the genus *Anopheles*, three species *An. stephensi, An. dthali*, and *An. superpictus* were identified. All three of these mosquitoes are among the main vectors of malaria in Iran.

An. stephensi: An important vector of human malaria throughout the Middle East and South Asia, including the Indian subcontinent and Pakistan, as well as through the Iran and Iraq to the Middle East and the Arabian Peninsula (Mehravaran *et al*., 2012). This species is also the main vector of malaria in the endemic malarious areas of southern Iran (Vatandoost *et al*., 2006) and has been reported from Ilam, Bushehr, Fars, Hormozgan, Kermanshah, Kerman, Kuhgiluyeh and Boyer-Ahmad, Khuzestan, Lorestan, Sistan-Baluchistan provinces. During spring and autumn, the population of *An.stephensi* has two main peaks of the seasonal activity.

In a study by Mehravaran *et al*. (2012), as well as Keshavarzi *et al*. (2017), the main peak in the population of this species occurred in May, and this agrees with our findings in Qir and Karzin County . Having enough information about the vectors' seasonal activity peaks can help to better plan and implement an effective vector control program during an epidemic event. This species is found in a wide range of natural and artificial habitats such as ponds, water holes, wells, irrigation canals, rice fields, and aquaculture ponds (Nikookar *et al*., 2016).

An. superpictus: is considered as a main malaria vector in the central plateau of Iran (Oshaghi *et al*., 2007) and a secondary malaria vector in the southern regions of the country (Soleimani-Ahmadi *et al*., 2015). This species has a wide distribution. Two different morphological forms (A and B) of this species have been reported from Iran (Oshaghi *et al*., 2007). In our study, this species was mostly collected in May and June. In a study conducted by Keshavarzi *et al*. (2017), the monthly population activity in Larestan county was reported from May to August and in Mohr city in April, May, July to September. A study in Kurdistan (western Iran) showed that *An. superpictus* has a frequency of 57.7%. It was the most abundant species of Anophelinae in this region and was active from July to October (Banafshi *et al*., 2013).

An. dthali: This species has spread in semi-arid regions from North Africa to northwestern Pakistan and southern Iran (Keshavarzi *et al*., 2017). In this study, among the three species of the genus *Anopheles*, this species had the highest frequency (40.84%). This species is a secondary vector of malaria in Hormozgan province, southern Iran (Vatandoost *et al*., 2006). Its monthly activity in Jask (a tropical city in Hormozgan province) has been reported all year round (Yeryan *et al*., 2016). In the study of Keshavarzi *et al*. (2017), its monthly activity in Darab (a city in Fars province) was from May to August (Keshavarzi *et al*., 2017). In our study, this species was reported in all months of sampling. This indicates that since our study area is a tropical region, this species is active in all warm months of the year.

A total of 8 species of the genus *Culex* were found in our study.

Cx. pipiens: This species is widespread in Europe, North America, Africa, some parts of Asia, South America and Australia (S. Nikookar *et al*., 2010; Vinogradova, 2000). The species is distributed in most parts of Iran (Aaim *et al*., 1985; S. Nikookar *et al*., 2010). Its monthly activity in Mazandaran province started at the end of May and increased in early July and decreased slowly in mid-summer (Nikookar *et al*., 2010). In the study of Keshavarzi *et al*. (2017), the activity of this species in Zarin Dasht (Fars province) is from June to September and in Mohr and Lamerd is from April to September (Keshavarzi *et al*., 2017). This species grows in ponds, drainage canals, animal footprints, riversides, holes and lake shores (Korba *et al*., 2016). In our study, this species was one of the most distributed species of the genus *Culex* and was collected from all sampling areas.

Cx. theileri: This species is found in the Palaearctic, Afrotropical and Oriental regions. It has been reported from all provinces of Iran (Aaim *et al*., 1985). This mosquito is a vector of *Setaria labiatopapillosa* (Alessandrini) and *Dirofilaria immitis* (Leydy) in Ardabil province (Azari-Hamidian, 2011). In our study, this species was caught from August to October. In the study of Keshavarzi *et al*. (2017), its activity in Darab and Mohr is from April to September. In a study on the biology of mosquitos in western Iran, it is mentioned that *Cx. theileri* prefers to live in larval habitats without vegetation and with stagnant, permanent, and clean water (Moosa-Kazemi *et al*., 2015). No larvae were collected from this species in this study and only adult stage of them were reported.

Cx. tritaeniorhynchus: The main vector of the encephalitis virus in Japan (Ramesh *et al*., 2015) and is limited to the Palaearctic (South Asia), Afrotropical and Oriental regions (Zaim & Cranston, 1986). A study in the Shadegan region (southwest of Iran) showed that this species is one of the most common species in the studied region (Navidpour *et al*., 2012). In a study by Ramesh *et al*. (2015), The main activity peak of this species was in the months of August to October. Similarly, in our study this species was collected mostly in August to October.

Cx. sinaiticus: This species has been reported from Bushehr, Khuzestan, Sistan- Baluchistan, Fars, Kerman and Hormozgan provinces (Aaim *et al*., 1985). In a study conducted by Keshavarzi *et al*. (2017) in Fars province, this species was one of the least populated mosquitoes with only 7 collected specimens. Similar research in Mahshar (Khuzestan province) showed that this species had the lowest frequency with a total of 4 specimens (Farhadinejad, Mousavi, & Amraee, 2015). Unlike other studies, this species has the highest larval frequency (36.9%) among all our samples. These results indicate that the geographical and climatic conditions are completely suitable for the growth and reproduction of this species in our study area. Furthermore, probably the lack of food competitors or possibly the lack of specific predators has allowed such an ideal development.

Cx. bitaeniorhynchus: This species has been reported from Kerman (Azari-Hamidian *et al*., 2005; Zaim, 1987), Sistan-Baluchistan, Fars, Bushehr, Hormozgan and Kohgiluyeh and Boyer-Ahmad provinces (Zaim, 1987). Its distribution is limited to the southern regions of Iran (Aaim *et al*., 1985). The larvae lives in breeding places with permanent or temporary water, stagnant or low flow, mainly in fresh and clear water, with vegetation and in fully or partial sun-lighted places (Zaim, 1987). In this study, only 3 adults were collected in September.

Cx. torrentium: It is often an ornithophilic species (Hesson, Östman, Schäfer, & Lundström, 2011; Hesson *et al*., 2014) and is widely distributed in the Holarctic and West Palaearctic and Central Asia. This species has been identified as a major vector of West Nile Virus (WNV) and Sindbis virus worldwide (Nikookar *et al*., 2016). It can transmit the Sindbis virus in Africa, India, Malaysia, Philippines, Australia and Sweden and is more effective in transmitting the virus than *Cx. pipiens* (Hesson *et al*., 2011;

Shaikevich, 2007). Human infections of Sindbis and West Nile Viruses have been reported in Iran especially the coastal areas of the Caspian Sea. A study in Mazandaran showed that this species is the most abundant mosquito among other species (Nikookar *et al*., 2016). In the present study, this species was identified only in larval stage form.

Cx. laticinctus: it is reported from Khorasan, Chaharmahal-Bakhtiari, Bushehr, Fars, Yazd (Zaim, 1987), Kerman (Azari-Hamidian *et al*., 2005; Zaim & Cranston, 1986), Kohgiluyeh and Boyer-Ahmad provinces. The larvae usually lives in permanent and vegetated water in fully or partial sun-lighted places (Zaim, 1987). In our study, this species had the highest frequency among the collected larval samples.

Only one species of *Culiseta* was identified in this study.

Cs. longiareolata: This species has been reported from all provinces of the country (Zaim & Cranston, 1986). In the study of Keshavarzi *et al*. (2017), 73 and 221 specimens of this species were obtained in Darab and Mohr (Fars province), respectively. It has been reported as the most abundant species in Kurdistan, Kermanshah and Sistan-Baluchistan provinces (Moosa-Kazemi *et al*., 2009; Moosa-Kazemi *et al*., 2015). In this study, this species was collected in September and October.

Among all the studied areas, Efzar had the highest species diversity (9 species out of 11). And the species of *Cx. bitaeniorhynchus* was reported only from this area. *Cx. theileri* and *Cx. laticinctus* were collected only from Efzar and Qir. While, *Cx. torrentium* and *Cx. tritaeniorhynchus* were observed only in Efzar and Qir regions.

Some species including *Cx. pipiens, Cx. sinaiticus, An. stephensi, An.dthali*, and *Cu. longiareolata* were collected from all four regions and showed the most distribution during all collected mosquitoes in this area.

Adults of some species such as *Cx. torrentium* and *Cx. tritaeniorhynchus* were not reported from any of the studied regions and only their larval stages were collected from Karzin and Emam Shahr areas. This phenomenon can be explained by the fact that these mosquitoes were probably exophilic species and their resting places were outside the human and animal shelters that we did not cover them. Therefore, adults of these species were not caught using the applied methods.

Only adult specimens of *An. superpictus*, a main vector of malaria in Iran, were collected from Emam Shahr and Efzar. However, other main malaria vectors (*An. stephensi* and *An. dthali*) were reported from all studied regions. This indicates that this species has a more limited range of distribution than other malaria vectors in this area.

Similar results were obtained for *Cu. longiareolata*. Only adult specimens were caught from all the studied areas and no larval samples were reported in our study. This phenomenon can probably be attributed to two main factors. One is that the larval habitats of this species are probably different from other species, and resulting with the applied sampling methods in the selected regions we could not catch the larvae. Also, our sampling methods or covered areas may not have been complete enough.

Conclusions

In this study, a total of 11 species in three genera were identified and reported based on the morphological traits of larvae and adults in Qir and Karzin County. In this relatively small region, compared to studies conducted in other parts of the province, there was considerable species diversity for mosquitoes (Keshavarzi *et al*., 2017; Soltani *et al*., 2017). This phenomenon is very important from an ecological and health point of view.

Due to the existence of some important and main vectors of diseases such as malaria, dengue fever, yellow fever, West Nile fever and other arboviral diseases in this region, the health system should always be alert about them. Continuous and accurate monitoring of these vectors should be included in the routine vector-borne diseases control program. In addition, due to the special climatic conditions of this area (being located in the tropical and Oriental region), if disease cases enter this region from other places (inside or outside the country), the possibility of an epidemic with high speed will be very probable.

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