

## Original Article

# Seasonal Activity of Adult Mosquitoes (Diptera: Culicidae) in a Focus of Dirofilariasis and West Nile Infection in Northern Iran

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## Abstract

**Background:** Mosquito-borne arboviruses such as West Nile, dengue, Rift Valley fever, and Sindbis viruses and the nematode *Dirofilaria* are reported in Iran, but there is little information on the seasonal activity of their vectors in the country. We aimed to determine the seasonal activity of adult mosquitoes (Diptera: Culicidae) in a focus of dirofilariasis and West Nile infection in Guilan Province, northern Iran.

**Methods:** Collections were carried out using light traps in seven counties at least two times from random sites and every two weeks from a fixed site (Pareh Village, Rudbar County) during Aug–Dec 2015 and Apr–Oct 2016.

**Results:** Overall, 16327 adult mosquitoes comprising 18 species representing seven genera were identified. The most prevalent species were *Cx. theileri* (23.59%), *Cx. tritaeniorhynchus* (20.75%), *Cx. pipiens* (19.37%), *Ae. vexans* (18.18%), *An. pseudopictus* (10.92%) and *An. maculipennis* s.l. (5.48%). *Aedes pulcritarsis* and *Cx. perexiguus* were found for the first time in Guilan Province. The active season of adult mosquitoes extended from early May to early Oct in the fixed site. There was no significant regression between the abundance of adult mosquitoes and the meteorological data during active season in the fixed site ( $P > 0.05$ ,  $R^2 = 0.31$ ).

**Conclusion:** Though no significant regression between the abundance of mosquitoes and the meteorological data was observed during active season, temperature and rice fields had a great influence in starting and ending active season in the region.

**Keywords:** *Dirofilaria*, *Flavivirus*, Flaviviridae, Vectors, Iran

## Introduction

West Nile virus (WNV) (Flaviviridae: *Flavivirus*) and its subtype Kunjin is distributed in Eurasia, Africa, North and Central America and Australia. Mosquitoes (Diptera: Culicidae), especially ornithophilic species, are the principal vectors of the virus and some virus isolations have been reported from soft and hard ticks. Wild birds, especially wetland species, are the principal vertebrate hosts, the virus has also been isolated from mammals and frogs (1, 2).

WNV infection is recorded from horses in

at least 26 provinces (out of total 31) in Iran (3–5), humans (6–12) and birds (13). Guilan Province in the Caspian Sea littoral of northern Iran, with vast wetlands, is one of the foci of WNV where infections are found in humans (1.4–10%) (4, 7–8), horses (2.2–25%) (3–4) and birds (especially the common coot, the main reservoir) (62.7%) (13). Recently, the virus was found in *Aedes (Ochlerotatus) caspius* (Pallas) s.l. [*Ochlerotatus caspius* s.l.] in West Azerbaijan Province, northwestern Iran, and

in *Cx. pipiens* Linnaeus in Guilan Province, northern Iran, respectively (14, 15).

Dirofilariasis is a disease caused by different species of the nematode genus *Dirofilaria* (Spirurida: Onchocercidae), especially *D. immitis* (canine or dog heartworm) and *D. repens*, transmitted by mosquitoes. The disease is cosmopolitan. The reservoirs of the nematodes are many different mammals, especially canids. Previously, human dirofilariasis (HD) was considered a rare disease, but at the present time, HD is classified as an emerging disease in some areas because the number of reported cases was dramatically increased (16).

Dirofilariasis is found in humans, dogs, wolves, jackals, foxes and cats in at least 15 provinces of Iran (17–22). Guilan Province is one of the foci of dirofilariasis, where *D. repens* infection is found in humans (17, 23) and *D. immitis* found in 4.4% (24) to 51.4% of local dogs (25, 26). *Culex theileri* Theobald is a known vector of *D. immitis* in northwestern Iran (27).

The last checklist of Iranian mosquitoes comprises 64 species and seven genera (28, 29). Subsequently, *Anopheles superpictus* Grassi includes two species in Iran based on the Internal Transcribed Spacer 2 (ITS2) sequences of rDNA (30), later listed as species A and B (31). A new species of the *Anopheles hyrcanus* group (*An. hyrcanus* spIR) was recognized from southwestern Iran, also based on ITS2 sequences (32). More recently, the occurrence of *Aedes* (*Stegomyia*) *albopictus* (Skuse) [*Stegomyia albopicta*] and *Ae.* (*Stg.*) *unilineatus* (Theobald) [*Stegomyia unilineata*] were reported in southeastern Iran and *Orthopodomyia pulcripalpis* (Rondani) in northern Iran, respectively (33–35). Overall, 30 species of mosquitoes representing seven genera were listed in Guilan Province (36).

A large amount of available data on mosquitoes in Iran is based on collections and ecology of larvae (27, 36–40 and many other references cited by aforementioned articles). Different methods of collecting adult mosquitoes,

such as using light traps, aspirators, pit shelters and total catch (Pyrethrum space spray), have been used mostly in relation to anopheline vectors of malarial protozoa (41–47). There are a few published documents in the country that deal with adult sampling, especially using light traps, which include culicines (27, 48–50), but there are no studies of seasonal activity. That is why there is very little information about the seasonal activity of culicine adults in Iran.

This study was carried out by means of light traps to study the seasonal activity of mosquitoes, especially probable and proven vectors of WNV and *Dirofilaria*, in Guilan Province, northern Iran.

## Materials and Methods

### Study area

Guilan Province locates in the Caspian Sea littoral of northern Iran, between the Caspian Sea and the Alborz Mountain range. It has coastal, plain, foothill, and mountainous areas with an area of approximately 14,700 square kilometers. The province is bordered by Mazandaran Province in the east, Ardabil Province in the west and Zanzan and Qazvin provinces in the south. It is also bordered by the Republic of Azerbaijan in the north as well as Russia across the Caspian Sea (Fig. 1). The province has a temperate climate and relatively warm-humid summer. It is located between 36°33'–38°27' N latitude and 48°32'–50°36' E longitude and formally includes 16 counties. Most areas of Guilan Province with about 1000–2000mm of rainfall annually, have the greatest amount of rainfall in Iran, and the main agricultural crop is rice. The province has vast deciduous forests of Hyrcania, temperate climate, vast wetlands and rice fields, which provide abundant habitats for mosquitoes.

### Specimen and data collection

In seven counties (including different topographical areas of the province) adult collections were carried out at least two times from

random (variable) sites during Aug–Dec 2015 and Apr–Oct 2016 (Fig. 1, Table 1). Sampling was also carried out at a fixed site (Pareh Village of Rudbar County, 36° 50.800' N, 49° 32.650' E, altitude 487m) every two weeks from Apr to Oct 2016. Rudbar County in southern Guilan has about 200–500mm annual rainfall and showed mountainous and less humid temperate climate similar Mediterranean Region. Pareh Village is in a foothill area and close to natural Hyrcanian forest and manmade woodland that includes trees such as olive, walnut, fig, Persian ironwood (*Parrotia persica*) and Caucasian elm or Caucasian zelkova (*Zelkova carpinifolia*). The main livelihood of the people in the village is husbandry and the main domestic animals are cattle and sheep. Dogs, horses, donkeys and fowls are also common animals in the region. The meteorological data of Pareh Village during 2016 is shown in Table 2. Two CDC light traps were used in each variable and fixed site. The light traps were suspended from the ceiling in animal shelters from sunset to sunrise, i.e. from 1800 to 0600 hrs. The electricity of traps was provided by 6-volt rechargeable batteries. Moreover, ad hoc collections were carried out using manual aspirators (hand catch) in the fixed and random sites. The specimens were identified using the morphological-based keys (29). The abbreviations of mosquito genera and subgenera follow Reinert (51). The specimens are deposited at the Museum of Medical and Veterinary Entomology, School of Health, Guilan University of Medical Sciences, Rasht, Iran.

### Determining species dominance structure

The dominance structure of a species is expressed as the percentage of specimens of the species in the whole sample. The following five percentage representation categories (52, 53) were used: Eudominant (ED) species (> 30%), dominant (D) (10–30%), subdominant (SD) (5–10%), recedent (R) (1–5%) and subrecedent (SR) (< 1%).

### Mapping collected mosquitoes and statistical analysis

ArcGIS 10.3 was used to create a geo-database of mosquitoes and to map the collection sites and the distributions of the most medically important species. The statistical analysis of mosquito abundance and meteorological data was carried out using the linear regression test of SPSS software (ver. 16 for Windows, SPSS Inc., Chicago, IL).

## Results

### Mosquito fauna

Overall, 16327 adult mosquitoes were collected during 29 surveys (Aug–Dec 2015 and Apr–Oct 2016): 15959 (97.75%) were captured using light traps and 368 (2.25%) by ad hoc hand catches (Table 3). Eighteen species representing seven genera were identified morphologically: *Anopheles* (*Anopheles*) *claviger* (Meigen), *An. (Ano.) hyrcanus* (Pallas), *An. (Ano.) maculipennis* Meigen s.l., *An. (Ano.) pseudopictus* Grassi, *An. (Cellia) superpictus*, *Aedes* (*Aedimorphus*) *vexans* (Meigen) [*Aedimorphus vexans*], *Ae. (Dahlia) geniculatus* (Olivier) [*Dahlia geniculata*], *Ae. (Ochlerotatus) caspius* s.l. [*Ochlerotatus caspius* s.l.], *Ae. (Och.) pulcritarsis* (Rondani) [*Oc. pulcritarsis*], *Coquillettidia* (*Coquillettidia*) *richiardii* (Ficalbi), *Cx. (Culex) mimeticus* Noè, *Cx. (Cux.) perexiguus* Theobald, *Cx. (Cux.) pipiens*, *Cx. (Cux.) theileri*, *Cx. (Cux.) tritaeniorhynchus* Giles, *Culiseta* (*Culiseta*) *annulata* (Schrank), *Orthopodomyia pulcripalpis* and *Uranotaenia* (*Pseudoficalbia*) *unguiculata* Edwards (Table 3). *Aedes pulcritarsis* and *Cx. perexiguus* were found for the first time in Guilan Province.

### Species dominance structure

Overall, 2734 anopheline adults (16.71%) and 13,593 culicine adults (83.29%) were collected. The most prevalent species were *Cx. theileri* (23.59%, dominant), *Cx. tritaeniorhynchus* (20.75%, dominant), *Cx. pipiens* (19.37%, dominant), *Ae. vexans* (18.18%, dominant), *An.*

*pseudopictus* (10.92%, dominant) and *An. maculipennis* s.l. (5.48%, subdominant). These six species included 16,054 specimens (98.1%) of the whole sample (Table 3). Moreover, they showed the widest distributions in the province (Table 4, Fig. 2). Regarding the dominance structure of subfamily Anophelinae, *An. pseudopictus* and *An. maculipennis* s.l. with the abundance percentages of 65.2% and 32.8% respectively, were both eudominant. In the case of percentage representation of subfamily Culicinae, *Cx. theileri* (28.34%), *Cx. tritaeniorhynchus* (24.93%), *Cx. pipiens* (23.27%) and *Ae. vexans* (21.84%) were dominant, as in the case of all mosquitoes, i.e. the total for both subfamilies.

### Seasonal activity and the fluctuations of rainfall and temperature

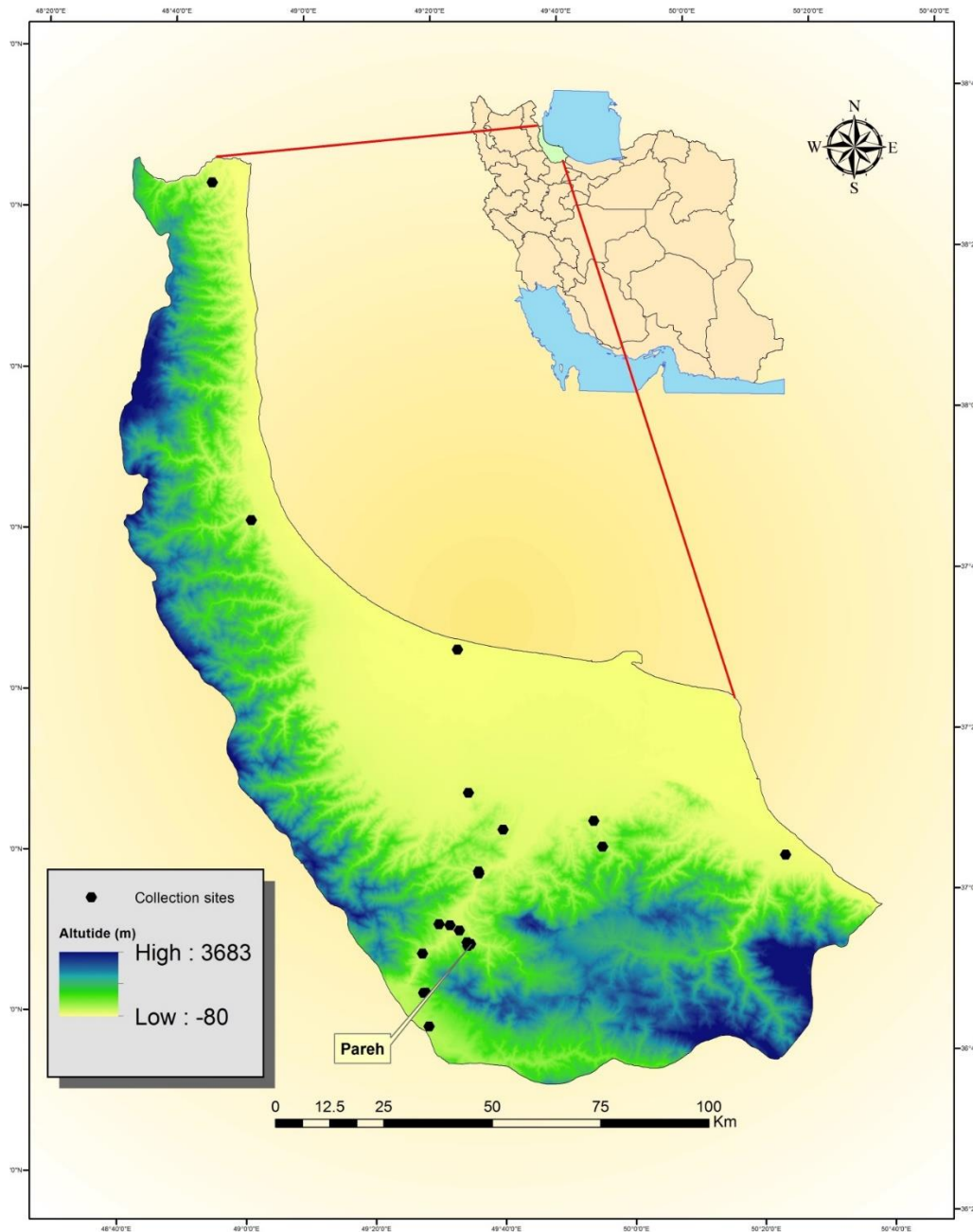
In general, the active season of adult mosquitoes extended from early May to early Oct in the fixed site (Pareh Village of Rudbar County). The peak of activity was late June for *Cx. theileri*, mid-July for *An. maculipennis* s.l., *An. pseudopictus* and *Cx. pipiens*, and late July for *Cx. tritaeniorhynchus*. While the peak of activity of most adult mosquitoes was late June to mid-July, and the abundance dramatically decreased after that, the monthly mean temperature increased by Aug. Also after Apr rainfall decreased in the fixed site during Jun and Jul and the rainy season started in Sep (Figs. 3–6). There is no significant regression between the abundance of adult mosquitoes and the meteorological data (Table 2) during active season in the fixed site ( $P > 0.05$ ,  $R^2 = 0.31$ ).

**Table 1.** Collection data for adult mosquitoes captures at variable sites in Guilan Province, Iran, August–December 2015 and April–October 2016

Locality (City/ Village)	Topography	County	Coordinates	Altitude (m)
Rostamabad	Plain	Rudbar	36° 52.999' N, 49° 29.385' E	215
Joben	Foothill	Rudbar	36° 53.072' N, 49° 27.658' E	399
Khaskool	Foothill	Rudbar	36° 50.789' N, 49° 32.669' E	470
Lafandsara	Foothill	Rudbar	36° 50.522' N, 49° 32.271' E	620
Rudbar	Foothill	Rudbar	36° 49.314' N, 49° 25.322' E	270
Klayeh	Foothill	Rudbar	36° 50.992' N, 49° 32.132' E	438
Rudabad	Plain	Rudbar	36° 52.397' N, 49° 30.871' E	192
Harkian	Foothill	Rudbar	36° 59.592' N, 49° 33.491' E	149
Siahroodposhteh	Foothill	Rudbar	36° 59.862' N, 49° 33.432' E	269
Upper Harzavil (Manjil)	Foothill	Rudbar	36° 44.495' N, 49° 26.072' E	506
Lower Harzavil (Manjil)	Foothill	Rudbar	36° 44.837' N, 49° 25.735' E	453
Halaj (Loshan)	Foothill	Rudbar	36° 40.306' N, 49° 26.792' E	307
Kacha	Foothill	Rasht	37° 05.173' N, 49° 36.973' E	124
Saghalaksar	Plain	Rasht	37° 09.596' N, 49° 31.334' E	53
Ghazian	Coastal	Anzali	37° 27.347' N, 49° 28.663' E	-21
Kandbon	Plain	Rudsar	37° 03.415' N, 50° 20.987' E	20
Kalesara	Foothill	Talish	37° 42.251' N, 48° 55.577' E	93
Eivazmahaleh	Foothill	Astara	38° 23.964' N, 48° 46.715' E	77
Sechekeh	Foothill	Siahkal	37° 06.755' N, 49° 50.985' E	205
Asooyebala (Tootaki)	Mountainous	Siahkal	37° 03.556' N, 49° 52.542' E	355

**Table 2.** The meteorological data of the fixed site (Pareh Village of Rudbar County), Guilan Province, Iran, 2016

Meteorological data	April	May	June	July	Aug	September	October	November
Maximum Temperature	20.88	26.71	30.00	29.68	33.53	27.94	20.64	14.91
Minimum Temperature	10.79	16.56	20.17	21.52	22.60	18.77	12.92	6.25
Average Temperature	15.84	21.64	25.08	25.60	28.06	23.36	16.78	10.58
Relative Humidity	69.06	67.04	59.58	65.86	56.53	67.63	72.65	67.39
Rainfall	40.34	17.89	10.73	36.95	2.22	56.71	58.87	73.30



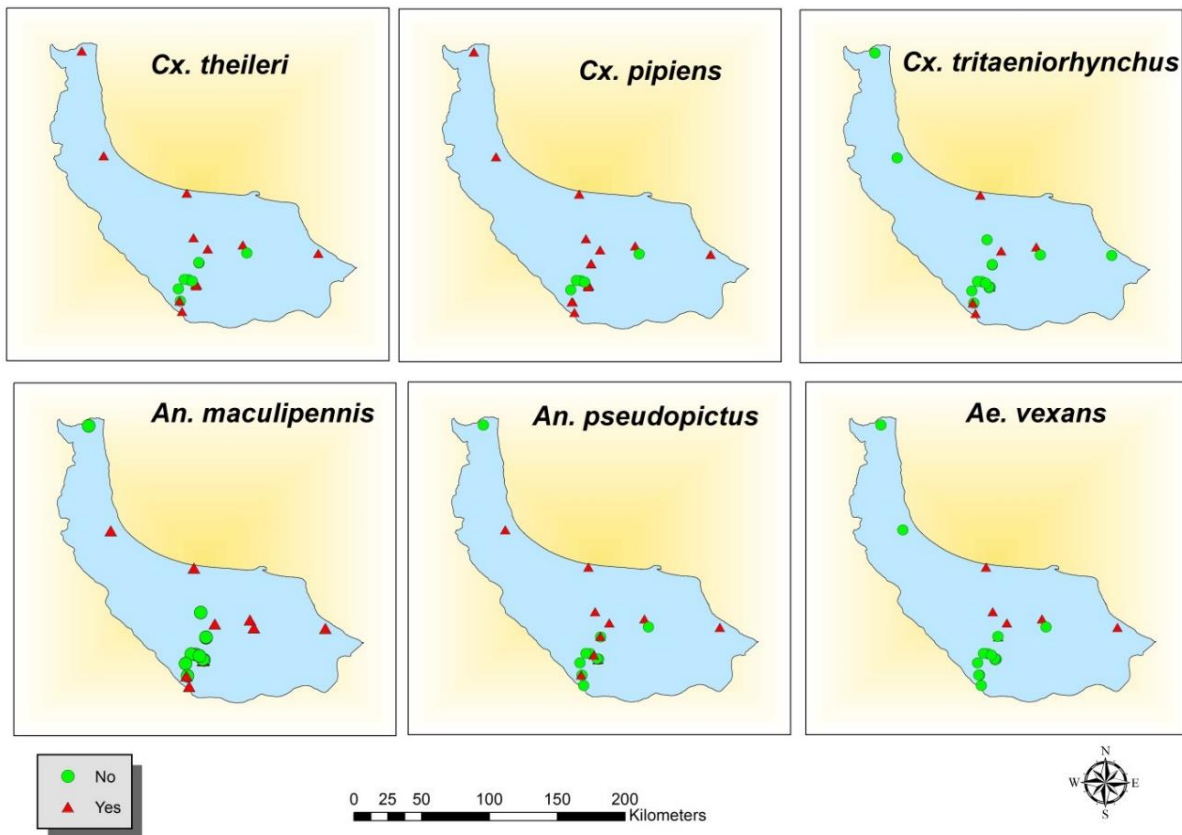
**Fig. 1.** Map of Iran highlighting the location of Guilan Province including mosquito collection sites surveyed in 2015–2016

**Table 3.** The collection method and composition of adult mosquitoes collected in Guilan Province, Iran, August–December 2015 and April–October 2016

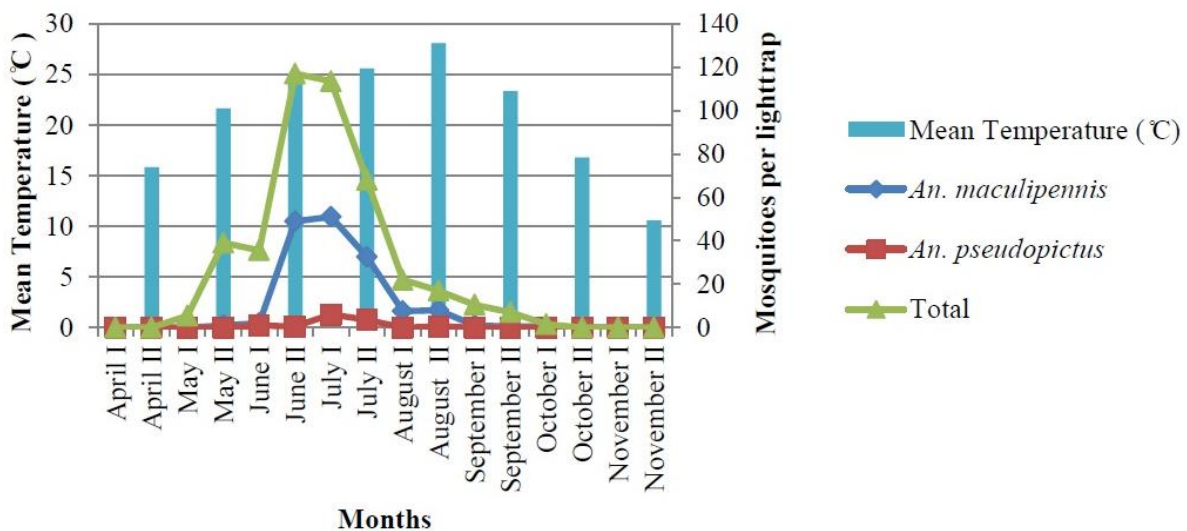
Species	Light trap		Hand catch		Total		Dominance structure
	n	%	n	%	n	%	
<i>An. claviger</i>	18	0.11	2	0.54	20	0.12	Subrecent
<i>An. hyrcanus</i>	29	0.18	-	-	29	0.17	Subrecent
<i>An. maculipennis</i> s.l.	752	4.71	144	39.13	896	5.48	Subdominant
<i>An. pseudopictus</i>	1760	11.03	23	6.25	1783	10.92	Dominant
<i>An. superpictus</i>	6	0.04	-	-	6	0.04	Subrecent
<i>Ae. caspius</i> s.l.	96	0.60	-	-	96	0.60	Subrecent
<i>Ae. geniculatus</i>	1	0.01	-	-	1	0.01	Subrecent
<i>Ae. pulcritarsis</i>	7	0.04	-	-	7	0.04	Subrecent
<i>Ae. vexans</i>	2930	18.36	39	10.59	2969	18.18	Dominant
<i>Cq. richiardii</i>	94	0.59	1	0.28	95	0.60	Subrecent
<i>Cx. mimeticus</i>	8	0.05	1	0.28	9	0.06	Subrecent
<i>Cx. perexiguus</i>	6	0.04	-	-	6	0.04	Subrecent
<i>Cx. pipiens</i>	3030	18.98	134	36.41	3164	19.37	Dominant
<i>Cx. theileri</i>	3844	24.09	9	2.44	3853	23.59	Dominant
<i>Cx. tritaeniorhynchus</i>	3375	21.15	14	3.80	3389	20.75	Dominant
<i>Cs. annulata</i>	1	0.01	-	-	1	0.01	Subrecent
<i>Or. pulcripalpis</i>	-	-	1	0.28	1	0.01	Subrecent
<i>Ur. unguiculata</i>	2	0.01	-	-	2	0.01	Subrecent
<b>Total</b>	<b>15959</b>	<b>100</b>	<b>368</b>	<b>100</b>	<b>16327</b>	<b>100</b>	

**Table 4.** The distribution of adult mosquitoes collected in different counties in Guilan Province, Iran, August–December 2015 and April–October 2016

Species	Locality						
	Rudbar	Rasht	Anzali	Rudsar	Talish	Astara	Siahkal
<i>An. claviger</i>	*	-	-	-	-	-	-
<i>An. hyrcanus</i>	-	*	-	*	-	-	-
<i>An. maculipennis</i> s.l.	*	*	*	*	*	-	*
<i>An. pseudopictus</i>	*	*	*	*	*	-	*
<i>An. superpictus</i>	*	-	-	-	-	-	-
<i>Ae. caspius</i> s.l.	*	-	*	-	-	-	-
<i>Ae. geniculatus</i>	*	-	-	-	-	-	-
<i>Ae. pulcritarsis</i>	*	-	-	-	-	-	*
<i>Ae. vexans</i>	*	*	*	*	-	-	*
<i>Cq. richiardii</i>	-	-	*	-	-	-	-
<i>Cx. mimeticus</i>	*	-	-	*	-	-	-
<i>Cx. perexiguus</i>	*	-	-	-	-	-	-
<i>Cx. pipiens</i>	*	*	*	*	*	*	*
<i>Cx. theileri</i>	*	*	*	-	-	-	*
<i>Cx. tritaeniorhynchus</i>	*	*	*	*	*	*	*
<i>Cs. annulata</i>	-	*	-	-	-	-	-
<i>Or. pulcripalpis</i>	*	-	-	-	-	-	-
<i>Ur. unguiculata</i>	*	-	-	-	-	-	-

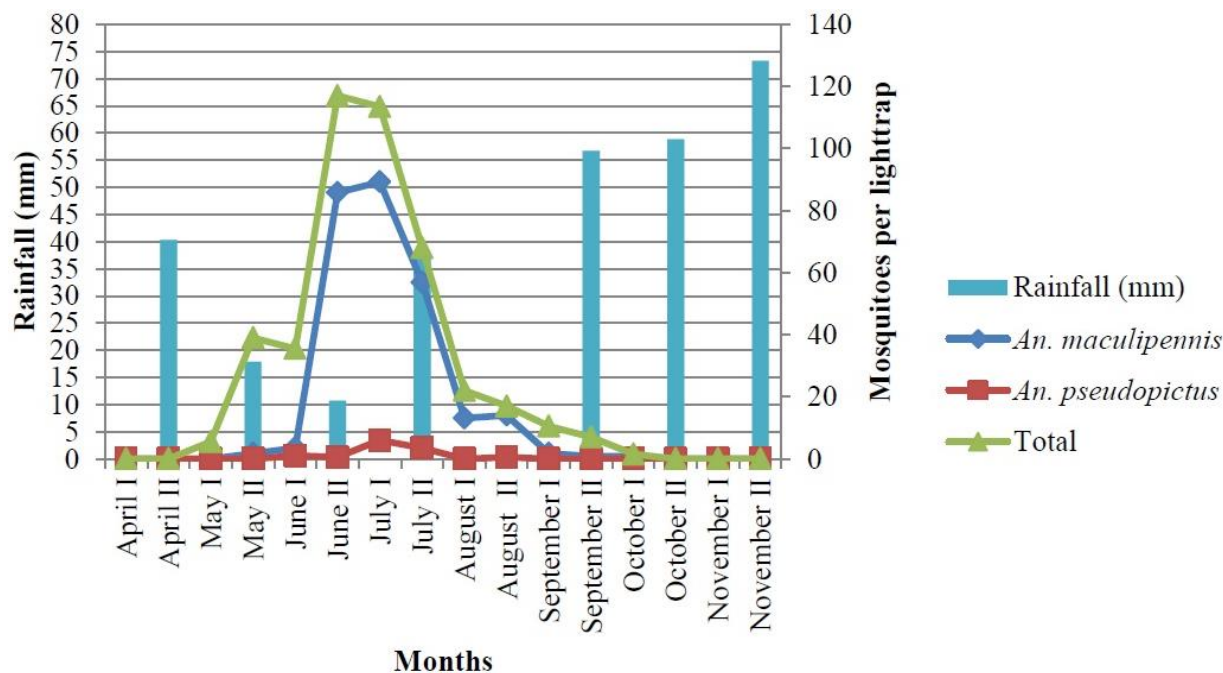


**Fig. 2.** Distribution map of the most prevalent and medically important mosquitoes in the study areas in Guilan Province, Iran, 2015–2016

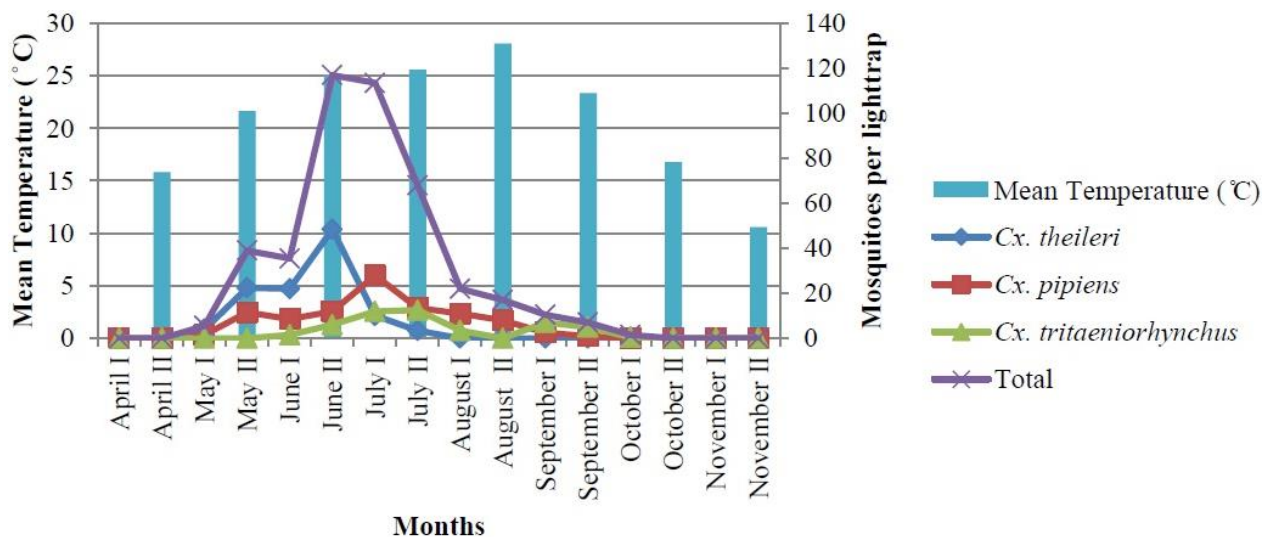


**Fig. 3.** Biweekly abundance of the most prevalent anopheline mosquitoes and monthly mean temperature in the fixed site, Parh Village of Rudbar County, Guilan Province, Iran, April–October 2016 (Total includes *Anopheles claviger*, *An. maculipennis* s.l., *An. pseudopictus*, *An. superpictus*, *Ae. pulcritarsis*, *Culex mimeticus*, *Cx. pipiens*, *Cx. theileri*, *Cx. tritaeniorhynchus*)



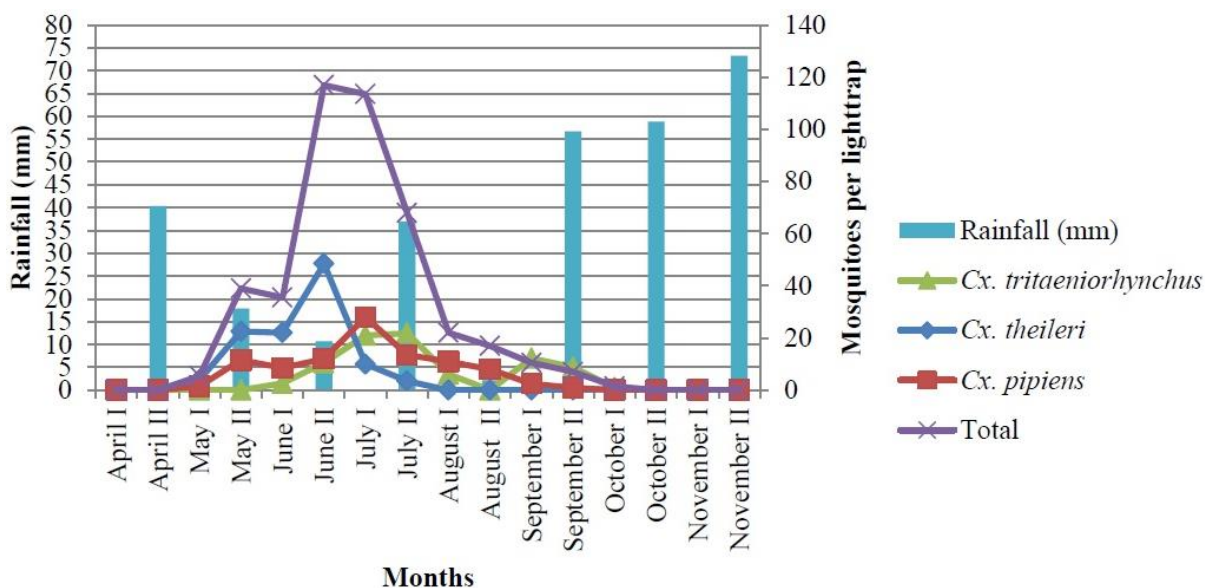


**Fig. 4.** Biweekly abundance of the most prevalent anopheline mosquitoes and monthly rainfall in the fixed site, Parih Village of Rudbar County, Guilan Province, Iran, April–October 2016 (Total includes *Anopheles claviger*, *An. maculipennis* s.l., *An. pseudopictus*, *An. superpictus*, *Ae. pulcritarsis*, *Culex mimeticus*, *Cx. pipiens*, *Cx. theileri*, *Cx. tritaeniorhynchus*)



**Fig. 5.** Biweekly abundance of the most prevalent culicine mosquitoes and monthly mean temperature in the fixed site, Parih Village of Rudbar County, Guilan Province, Iran, April–October 2016 (Total includes *Anopheles claviger*, *An. maculipennis* s.l., *An. pseudopictus*, *An. superpictus*, *Ae. pulcritarsis*, *Culex mimeticus*, *Cx. pipiens*, *Cx. theileri*, *Cx. tritaeniorhynchus*)





**Fig. 6.** Biweekly abundance of the most prevalent culicine mosquitoes and monthly rainfall in the fixed site, Parih Village of Rudbar County, Guilan Province, Iran, April–October 2016 (Total includes *Anopheles claviger*, *An. maculipennis* s.l., *An. pseudopictus*, *An. superpictus*, *Ae. pulcritarsis*, *Culex mimeticus*, *Cx. pipiens*, *Cx. theileri*, *Cx. tritaeniorhynchus*)

## Discussion

### Mosquito fauna

During the present investigation, 18 species representing seven genera of mosquitoes were collected in Guilan Province in which *Ae. pulcritarsis* and *Cx. perexiguus* were new records for the province. Thus, the number of species recorded in the province increased from 30 (36) to 33 (35 and the present investigation).

Seven species, *An. (Ano.) plumbeus* Stephens, *Ae. (Dah.) echinus* (Edwards) [*Dahlia echinus*], *Cx. (Maillotia) hortensis* Ficalbi, *Cx. (Cux.) torrentium* Martini, *Cx. (Neoculex) territans* Walker, *Cs. (Allotheobaldia) longiareolata* (Macquart) and *Cs. (Culicella) morsitans* (Theobald) whose larvae were collected during recent years in Guilan Province (36), were not captured in the present study. The reason is the rarity of some aforementioned species and the tendency of some, such as *Cx. hortensis*, *Cx. territans* and *Cs. morsitans*, to feed on birds, amphibians or reptiles (54), thus they were not attracted to light traps used in animal (cattle and sheep) shelters during the study.

Also, there are seven species of the Maculipennis Group in Guilan Province differentiated by egg patterns or the polymerase chain reaction (PCR) technique (36), which could not be differentiated with the morphology-based keys of females and larvae (29) used in the present study.

Among the species collected, *Ae. caspius* s.l. is known to vector WNV in West Azerbaijan Province in northwestern Iran (14). Shahhosseini et al. (55) referred to the virus later as Kunjin-related West Nile Virus. Moreover, WNV was found in *Cx. pipiens* in the Sepid-Rud valley of Guilan Province (15). In addition, *An. maculipennis* s.l., *Ae. vexans*, *Cq. richiardii*, *Cx. perexiguus*, *Cx. theileri*, *Cx. tritaeniorhynchus* and *Ur. unguiculata* is believed to play role as vectors of WNV in different countries of the Old World (1, 56). Among the aforementioned species, *Cq. richiardii* and *Cx. pipiens* in Europe and *Cx. tritaeniorhynchus* in Asia are the main vectors of

the virus (1).

*Culex theileri* has been found to be the vector of *Dirofilaria*, the causal agent of dirofilariasis, in Ardebil Province in northwestern Iran (27), and *An. claviger*, *An. hyrcanus*, *An. maculipennis*, *An. pseudopictus*, *An. superpictus*, *Ae. vexans*, *Ae. caspius*, *Ae. geniculatus*, *Cq. richiardii*, *Cx. pipiens*, *Cx. tritaeniorhynchus*, *Cs. annulata* and *Ur. unguiculata* are known the vectors of *Dirofilaria* in different countries of the western Palaearctic Region (54, 57–59).

### Species dominance structure

In the present study, the most abundant species were *Cx. theileri*, *Cx. tritaeniorhynchus*, *Cx. pipiens*, *Ae. vexans*, *An. pseudopictus* and *An. maculipennis* s.l., respectively. With the exception of *An. maculipennis* s.l., which is subdominant, they are all dominant according to the classification (52, 53) (Table 3). This is concordant with the previous findings in the province based on collections of larvae (36–38), as well as adults (37, 60). The exception is *Cx. theileri*. The species had been found less often in the larval stage than any other *Culex* in the province (38). One reason is probably due to sampling, the heavy rainfall in the province results in a great number of different natural larval habitats that are favorable for *Cx. theileri* (38), but those habitats are not easily located and sampled. On the other hand, the favorable larval habitats of some species, such as *Cx. pipiens* and *Cx. tritaeniorhynchus*, i.e. artificial containers and rice fields, respectively, are easier to find and sample. Another reason is probably the biology of the species, some species such as *Cx. hortensis* and *Cx. territans*, which do not bite humans and mammals but mostly feed on amphibians, reptiles or birds (54), have been collected very often as larvae (38). They were not collected during the present investigation by means of aspirators and light traps from animal shelters which attract *Cx. theileri* (Table 3). The most prevalent species of the province, *An. maculipennis* s.l., *An.*

*pseudopictus*, *Ae. vexans*, *Cx. pipiens*, *Cx. theileri* and *Cx. tritaeniorhynchus* are known vectors of both WNV and *Dirofilaria* (1, 57, 59).

### Seasonal activity and the fluctuations of rainfall and temperature

During the present investigation, *Ae. vexans* was one of most abundant species and most prevalent aedine species (Table 3), as noted previously (37, 60). However, most specimens were collected from Anzali (Table 4) and the species was not collected from the fixed site, so its seasonal activity is not discussed here. *Anopheles maculipennis* s.l. showed the peak of activity in the mid-Jul (Figs. 3, 4). The peak of monthly activity of anophelines (including *An. maculipennis* s.l. and *An. superpictus*) was reported during Jul–Aug in Kalaleh County of Golestan Province, northern Iran (47). The most *An. maculipennis* s.l. was captured in Aug in Aras Valley, Turkey, adjoining Iran (61). There are no records for the seasonal activity of culicine adults in Iran. The peak of activity of *Cx. pipiens* was found in Jul in northern Italy (62). That is in concordance with the result of the present study (Figs. 5, 6). However, the peak of activity of *Cx. pipiens* was recorded in Aug in the Belek Region and Aras Valley of Turkey (61, 63). Most *Cx. theileri* was found in Jun in Aras Valley of Turkey (61), which is similar to the present study (Figs. 5, 6), however, the peak activity of this species was reported in Aug in Ankara, Turkey (64). Moreover, most *Cx. tritaeniorhynchus* was captured in Aug in Belek Region of Turkey (63), while the peak of activity was observed in Jul in the present study (Figs. 5, 6). Differences between the results of the present investigation and the findings in other regions may be explained by differences in the topography and climates (especially temperature) which influence the bionomics of mosquitoes. On the other hand, some differences are due to sampling regimes. For example, mosquito abundance was reported based on weekly catches (62), and on monthly catches (61, 63, 64), whereas the seasonal

activity is based on biweekly captures in the present study and another study (47). Besides, dry-ice baited traps was used for sampling by Alten et al. (63), BG-traps by Roiz et al. (62) and spray sheet collections by Sofizadeh et al. (47), whereas light traps were used by Simsek (64), Alkan and Aldemir (61) and in this study.

Though the mean monthly temperature of about 16 °C is a limiting factor in the activity of adult mosquitoes in the study area (Figs. 3, 5), no significant regression was observed between different meteorological data (Table 2) and the abundance of adult mosquitoes during active season ( $P > 0.05$ ,  $R^2 = 0.31$ ). During the present study, the peak of activity of most adult mosquitoes was late Jun to mid-Jul, only the peak of activity of *Cx. tritaeniorhynchus* was in late Jul (Figs. 3–6), after which the abundance dramatically decreased as temperature increased by Aug (Figs. 3, 5). High temperature ( $> 35$  °C) is generally a limiting factor for the abundance of adult mosquitoes, especially in localities with warm climate such as southern Iran (41) and Saudi Arabia (65). However, it does not seem that temperature was a key factor in decreasing the abundance of adult mosquitoes in the area of the present study, because the temperature does not exceed 34 °C and the monthly mean temperature is lower than 30 °C in the fixed site in Aug (Figs. 3, 5). In addition, rainfall decreased in the fixed site during Jun and Jul while the abundance increased. The rainy season started in Sep while the abundance of mosquitoes dramatically decreased (Figs. 4, 6). A key factor mentioned here is rice fields, the main larval habitats of the most prevalent species, are dry during Aug. Temperature decreases significantly during Sep and Oct, consequently, the prevalence of mosquitoes decreases.

In view of integrated vector management, ecological data, especially seasonal activity, is very important for intervention measurements. On the other hand, one of main intervention measurements is using pesticides yet. There is little-published data about the susceptibility sta-

tus of mosquitoes, especially culicines (66, 67), in northern Iran. This subject can be a goal for forthcoming studies in Guilan Province.

## Conclusion

Though there was no significant regression between the abundance of adult mosquitoes and the meteorological data in the fixed site during active season, temperature and rice fields had a great influence in starting and ending active season in the region. The seasonal activity of the important species *Ae. vexans*, other species found less abundant in this study, host preference analysis and filarial and arbovirus screening should be the subjects of future investigations in the region.

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## References

- Hubálek Z, Halouzka J (1999) West Nile fever—a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis*. 5: 643–650.
- Medlock JM, Snow KR, Leach S (2005) Potential transmission of West Nile virus in the British Isles: an ecological review of candidate mosquito bridge vectors. *Med Vet Entomol*. 19: 2–21.
- Ahmadnejad F, Otarod V, Fallah MH, Lowenski S, Sedighi-Moghaddam R, Zavareh A, Durand B, Lecollinet S, Sabatier P (2011) Spread of West Nile virus in Iran: a cross-sectional serosurvey in equines, 2008–2009. *Epidemiol Infect*. 139: 1587–1593.
- Chinikar S, Shah-Hosseini N, Mostafavi E, Moradi M, Khakifirouz S, Jalali T, Goya MM, Shirzadi MR, Zainali M, Fooks AR (2013) Seroprevalence of West Nile virus in Iran. *Vector-Borne Zoonotic Dis*. 13: 586–589.
- Pourmahdi Borujeni M, Ghadrhan Mashadi AR, Seifi Abad Shapouri MR, Zeinvand M (2013) A serological survey on antibodies against West Nile virus in horses of Khuzestan Province. *Iran J Vet Med*. 7: 185–191.
- Naficy K, Saidi S (1970) Serological survey on viral antibodies in Iran. *Trop Geogr Med*. 22(2): 183–188.
- Saidi S (1974) Viral antibodies in preschool children from the Caspian area, Iran. *Iran J Publ Health*. 3: 83–91.
- Saidi S, Tesh R, Javadian E, Nadim A (1976) The prevalence of human infection of West Nile in Iran. *Iran J Publ Health*. 5: 8–14.
- Sharifi Z, Mahmoodian M, Talebian A (2010) A Study of West Nile Virus Infection in Iranian Blood Donors. *Arch Iran Med*. 13: 1–4.
- Chinikar S, Javadi A, Ataei B, Shakeri H, Moradi M, Mostafavi E, Ghiasi SM (2012) Detection of West Nile virus genome and specific antibodies in Iranian encephalitis patients. *Epidemiol Infect*. 140: 1525–1529.
- Shah-Hosseini N, Chinikar S, Ataei B, Fooks AR, Groschup MH (2014) Phylogenetic analysis of West Nile virus genome, Iran. *Emerg Infect Dis*. 20: 1419–1421.
- Meshkat Z, Chinikar S, Shakeri MT, Manavifar L, Moradi M, Mirshahabi H, Jalali T, Khakifirouz S, Shahhosseini N (2015) Prevalence of West Nile virus in Mashhad, Iran: A population-based study. *Asian Pac J Trop Med*. 8: 203–205.
- Fereidouni SR, Ziegler U, Linke S, Niedrig M, Modirrousta H, Hoffmann B, Groschup H (2011) West Nile virus monitoring in migrating and resident water birds in Iran: are common coots the main reservoirs of the virus in wetlands? *Vector-Borne Zoonotic Dis*. 11: 1377–1381.
- Bagheri M, Terenius O, Oshaghi MA, Motazakker M, Asgari S, Dabiri F, Vatandoost H, Mohammadi Bavani M, Chavshin AR (2015) West Nile virus in mosquitoes of Iranian wetlands. *Vector-Borne Zoonotic Dis*. 15: 750–754.
- Shahhosseini N, Chinikar S, Moosa-Kazemi SH, Sedaghat MM, Kayedi MH, Luhken R, Schmidt-Chanasit J (2017) West Nile Virus lineage-2 in *Culex* specimens from Iran. *Trop Med Int Health*. 22: 1343–1349.
- Simon F, Siles-Lucas M, Morchon R, Gonzalez-Miguel J, Mellado I, Carreton E, Montoya-Alonso JA (2012) Human and animal dirofilariasis: the emergence of a zoonotic mosaic. *Clin Microbiol Rev*. 25: 507–544.
- Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E, Mobedi I, Abai MR (2007)

- Review of dirofilariasis in Iran. J Med Fac Guilan Univ Med Sci. 15(60): 102–113 (in Persian with English abstract).
18. Ranjbar-Bahadori S, Hekmatkhah A (2007) A study on filariosis of stray dogs in Garmsar. J Vet Res. 62: 73–76.
  19. Akhtardanesh B, Radfar MH, Voosough D, Darijani N (2011) Seroprevalence of canine heartworm disease in Kerman, southeastern Iran. Comp Clin Pathol. 20: 573–577.
  20. Khedri J, Radfar MH, Borji H, Azizzadeh M, Akhtardanesh B (2014) Canine heartworm in [southeastern] of Iran with review of disease distribution. Iran J Parasitol. 9: 560–567.
  21. Heidari Z, Kia EB, Arzamani K, Sharifdini M, Mobedi I, Zarei Z, Kamranrashani B (2015) Morphological and molecular identification of *Dirofilaria immitis* from jackal (*Canis aureus*) in North Khorasan, northeast Iran. J Vector Borne Dis. 52: 329–333.
  22. Mirahmadi H, Maleki A, Hasanzadeh R, Bagher Ahoo M, Mobedi I, Rostami A (2017) Ocular dirofilariasis by *Dirofilaria immitis* in a child in Iran: a case report and review of the literature. Parasitol Int. 66: 978–981.
  23. Ashrafi K, Golchai J, Geranmayeh S (2010) Human subcutaneous dirofilariasis due to *Dirofilaria (Nochtiella) repens*: clinically suspected as cutaneous fascioliasis. Iran J Public Health. 39: 105–109.
  24. Ranjbar-Bahadori S, Veshgini A, Shirani D, Eslami A, Mohieddin H, Shemshadi B, Masooleh R (2010) Epidemiological aspects of canine dirofilariasis in the north of Iran. Iran J parasitol. 6: 73–80.
  25. Hosseini SH, Malmasi A, Aramon M (2010) Comparison of two diagnostic methods for *Dirofilaria immitis*: modified Knott test and ELISA. J Vet Med Lab. 2: 87–96 (in Persian with English abstract).
  26. Malmasi A, Hosseini SH, Aramoon M, Bahonar A, Seifi HA (2011) Survey of canine *Dirofilaria immitis* infection in Caspian provinces of Iran. Iran J Vet Res. 12: 340–344.
  27. Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E, Abai MR, Mobedi I, Linton YM, Harbach RE (2009) Distribution and ecology of mosquitoes in a focus of dirofilariasis in northwestern Iran, with the first finding of filarial larvae in naturally infected local mosquitoes. Med Vet Entomol. 23: 111–121.
  28. Azari-Hamidian S (2007) Checklist of Iranian mosquitoes (Diptera: Culicidae). J Vector Ecol. 32(2): 235–242.
  29. Azari-Hamidian S, Harbach RE (2009) Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). Zootaxa. 2078: 1–33.
  30. Oshaghi MA, Yaghoobi-Ershadi MR, Shemshad Kh, Pedram M, Amani H (2008) The *Anopheles superpictus* complex: introduction of a new malaria vector complex in Iran. Bull Soc Pathol Exot. 101: 429–434.
  31. Harbach RE (2013) The Phylogeny and Classification of *Anopheles*. In: Manguin S (Ed). *Anopheles Mosquitoes - New Insights into Malaria Vectors*. InTech, Rijeka, Croatia, pp. 1–55.
  32. Djadid ND, Jazayeri H, Gholizadeh S, Rad ShP, Zakeri S (2009) First record of a new member of *Anopheles* Hyrcanus Group from Iran: molecular identification, diagnosis, phylogeny, status of kdr resistance and Plasmodium infection. J Med Entomol. 46: 1084–1093.
  33. Doosti S, Yaghoobi-Ershadi MR, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Gooya MM, Vatandoost H, Shirzadi MR, Mostafavi E (2016) Mosquito surveillance and the first record of the invasive mosquito species *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) in southern Iran. Iran J Public Health. 45(8): 1064–1073.
  34. Yaghoobi-Ershadi MR, Doosti S, Schaffner

- F, Moosa-Kazemi SH, Akbarzadeh K, Yaghoobi-Ershadi N (2017) Morphological studies on adult mosquitoes (Diptera: Culicidae) and first report of the potential Zika Virus vector *Aedes (Stegomyia) unilineatus* (Theobald, 1906) in Iran. *Bull Soc Pathol Exot.* 110: 116–121.
35. Azari-Hamidian S, Norouzi B, Noorollahi A (2017) *Orthopodomyia pulcripalpis* (Diptera: Culicidae), a genus and species new to the Iranian mosquito fauna, with a review of biological information. *Zootaxa.* 4299: 141–145.
  36. Azari-Hamidian S (2011) Larval habitat characteristics of the genus *Anopheles* (Diptera: Culicidae) and a checklist of mosquitoes in Guilan Province, northern Iran. *Iran J Arthropod-Borne Dis.* 5: 37–53.
  37. Azari-Hamidian S, Joeafshani MA, Rassaei A, Mosslem M (2002) Mosquitoes of the genus *Aedes* (Diptera: Culicidae) in Guilan. *J Med Fac Guilan Univ Med Sci.* 11: 29–39 (in Persian with English abstract)
  38. Azari-Hamidian S (2007) Larval habitat characteristics of mosquitoes of the genus *Culex* (Diptera: Culicidae) in Guilan Province, Iran. *Iran J Arthropod-Borne Dis.* 1: 9–20.
  39. Nikookar SH, Moosa-Kazemi SH, Oshaghi MA, Vatandoost H, Yaghoobi-Ershadi MR, Enayati AA, Motevali-Haghi F, Ziapour SP, Fazeli-Dinan M (2015) Biodiversity of culicid mosquitoes in rural Neka township of Mazandaran Province, northern Iran. *J Vector Borne Dis.* 52: 63–72.
  40. Nikookar SH, Fazeli-Dinan M, Azari-Hamidian S, Mousavinasab SN, Arabi M, Ziapour SP, Shojaee J, Enayati A (2017) Species composition and abundance of mosquito larvae in relation with their habitat characteristics in Mazandaran Province, northern Iran. *Bull Entomol Res.* 107: 598–610.
  41. Zaim M, Ershadi MRY, Manouchehri AV, Hamdi MR (1986) The use of CDC light traps and other procedures for sampling malaria vectors in southern Iran. *J Am Mosq Control Assoc.* 2: 511–515.
  42. Zaim M, Subbarao SK, Manouchehri AV, Cochrane AH (1993) Role of *Anopheles culicifacies* s.l. and *An. pulcherrimus* in malaria transmission in Ghassreghand (Baluchistan), Iran. *J Am Mosq Control Assoc.* 9: 23–26.
  43. Zaim A, Manouchehri AV, Motabar M, Emadi AM, Nazari M, Pakdad K, Kayedi MH, Mowlaii G (1995) *Anopheles culicifacies* in Baluchistan, Iran. *Med Vet Entomol.* 9: 181–186.
  44. Basseri H, Raeisi A, Ranjbar Khakha M, Pakarai A, Abdolghafar H (2010) Seasonal abundance and host-feeding patterns of anopheline vectors in malaria endemic area of Iran. *J Parasitol Res.* 2010: 671291.
  45. Oshaghi MA, Vatandoost H, Gorouhi A, Abai MR, Madjidpour A, Arshi S, Sadeghi H, Nazari M, Mehravaran A (2011) Anopheline species composition in borderline of Iran-Azarbaijan. *Acta Trop.* 119: 44–49.
  46. Maghsoodi N, Ladonni H, Basseri HR (2015) Species composition and seasonal activities of malaria vectors in an area at reintroduction prevention stage, Khuzestan, south-western Iran. *J Arthropod Borne Dis.* 9: 60–70.
  47. Sofizadeh A, Edalat H, Abai MR, Hanafi-Bojd AA (2016) Fauna and some biological characteristics of *Anopheles* mosquitoes (Diptera: Culicidae) in Kalaleh County, Golestan Province, northeast of Iran. *Asia Pac J Trop Biomed.* 6: 730–734.
  48. Ghavami MB, Ladonni H (2006) The fauna and frequency of different mosquito species (Diptera: Culicidae) in Zanzan Province. *J Zanzan Univ Med Sci.* 13(53): 46–54 (in Persian with English abstract).
  49. Navidpour S, Vazirianzadeh B, Harbach R,

- Jahanifard E, Moravvej SA (2012) The identification of culicine mosquitoes in the Shadegan wetland in southwestern Iran. *J Insect Sci.* 12: 105.
50. Soltani Z, Keshavarzi D, Ebrahimi M, Soltani A, Moemenbellah-Fard MJ, Soltani F, Faramarzi H, Amraee K, Elyasigomari A (2017) The fauna and active season of mosquitoes in west of Fars Province, southwest of Iran. *Arch Razi Inst.* 72: 203–208.
  51. Reinert JF (2009) List of abbreviations for currently valid generic-level taxa in family Culicidae (Diptera). *Eur Mosq Bull.* 27: 68–76.
  52. Tischler W (1949) Grundzüge der terrestrischen Tierökologie. Friedrich Vieweg and Sohn Braunschweig.
  53. Heydemann B (1955) Die Frage der topographischen Übereinstimmung des Lebensraumes von Pflanzen- und Tiergesellschaften. *Verh Dtsch Zool Ges Erlangen.* 19: 444–452.
  54. Gutsevich AV, Monchadskii AS, Shtakelberg AA (1974) Fauna of the USSR Diptera Volume III No4 Mosquitoes Family Culicidae. *Akad Nauk SSSR Zool Inst NS No 100, Leningrad* (in Russian, English translation by Lavooott R, edited by Theodor O, Israel Program for Scientific Translations, Jerusalem).
  55. Shahhosseini N, Chinikar S (2016) Genetic evidence for circulation of Kunjin-related West Nile virus strain in Iran. *J Vector Borne Dis.* 53: 384–386.
  56. Pachler K, Lebl K, Berer D, Rudolf I, Hubálek Z, Nowotny N (2014) Putative new West Nile virus lineage in *Uranotaenia unguiculata* mosquitoes, Austria, 2013. *Emerg Infect Dis.* 20: 2119–2122.
  57. Ludham KW, Jachowski LA Jr, Otto GF (1970) Potential vectors of *Dirofilaria immitis*. *J Am Vet Med Assoc.* 157(10): 1354–1359.
  58. Anderson RC (2000) Nematod Parasites of Vertebrates. Their Development and Transmission. 2nd ed. CABI Publishing, Wallingford.
  59. Şuleşco T, von Thien H, Toderas L, Toderas I, Lühken R, Tannich E (2016) Circulation of *Dirofilaria repens* and *Dirofilaria immitis* in Moldova. *Parasit Vectors.* 9(1): 627.
  60. Azari-Hamidian S, Joeafshani MA, Mosslem M, Rassaei AR (2003) Adult mosquito habitats and resting-places in Guilan Province (Diptera: Culicidae). *Hakim.* 6: 55–62.
  61. Alkan SS, Aldemir A (2010) Seasonal dynamics of mosquitoes (Diptera: Culicidae) in animal barns and houses in Aras Valley, Turkey. *Kafkas Univ Vet Fak Derg.* 16: 43–48.
  62. Roiz D, Vasquez A, Rosa R, Munoz J, Arnoldi D, Rosso F, Fiuerola J, Tenorio A, Rizzoli A (2012) Blood meal analysis, flavivirus screening, and influence of meteorological variables on the dynamics of potential mosquito vectors of West Nile virus in northern Italy. *J Vector Ecol.* 37(1): 20–28.
  63. Alten B, Bellini R, Caglar SS, Simsek FM, Kaynas S (2000) Species composition and seasonal dynamics of mosquitoes in the Belek region of Turkey. *J Vector Ecol.* 25: 146–154.
  64. Simsek FM (2004) Seasonal larval and adult population dynamics and breeding habitat diversity of *Culex theileri* Theobald, 1903 (Diptera: Culicidae) in the Golbasi District, Ankara, Turkey. *Turk J Zool.* 28: 337–344.
  65. Alahmad AM (2012) Mosquito fauna (Diptera: Culicidae) of the eastern region of Saudi Arabia and their seasonal abundance. *J King Saud Univ.* 24: 55–62.
  66. Ataie A, Moosa-Kazemi SH, Vatandoost H, Yaghoobi-Ershadi MR, Bakhshi H, Anjomruz M (2015) Assessing the susceptibility status of mosquitoes (Diptera: Culicidae) in a dirofilariasis focus, north-western Iran. *J Arthropod-Borne Dis.* 9:



7–21.

67. Ghorbani F, Vatandoost H, Hanafi-Bojd AA, Abai MR, Nikookar H, Enayati AA (2018) High resistance of vector of West Nile virus, *Culex pipiens* Linnaeus (Diptera: Culicidae) to different insecticides recommended by WHO in northern Iran. J Arthropod-Borne Dis. 12: 24–30.