

## Diversity and species composition of mosquitoes (Culicidae: Diptera) in Noor County, northern Iran

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**Abstract.** Mosquitoes are highly important as public health problem due to their blood sucking habits and transmitting malaria, arboviruses and other diseases to humans. The present research was undertaken to determine the fauna, abundance, monthly distribution and activity of Culicidae mosquito larvae and adults in Noor County, northern Iran. This cross-sectional, descriptive study was conducted from August 2012 to November 2013 in the rural and urban outskirts of Noor. In each area, natural larval breeding places such as river beds, shallow wells, pits, sewer, marsh, small holes, tracks of animals as well as man-made breeding places like pools and rice paddy fields have been visited monthly for larval collection. To collect adult mosquitoes, human and animal dwellings including bedrooms, store rooms, toilets, barns, stables and pen were surveyed using WHO standard methods. A total of 844 larvae and 1484 adult mosquitoes were caught. Of the 665 Culicinae larvae, 501 were *Cx. pipiens* (75.3%), 108 *Cx. mimeticus* (16.2%) and 56 *Cx. theileri* (8.4%). Of the 179 Anophelinae larvae, 96 were *An. hyrcanus* (53.6%) and 83 *An. maculipennis s.l.* (46.4%). Among 889 adult Culicinae, 495 were *Cx. pipiens* (55.7%), 238 *Cx. mimeticus* (26.8%), 156 *Cx. theileri* (17.6%), and from 595 adult Anophelinae 371 were *An. hyrcanus* (62.4%) and 224 *An. maculipennis* (37.7%). *Anopheles hyrcanus* with 96 larvae (53.6%) and 371 adults (62.4%) and *Cx. pipiens* with 501 larvae (75.3%) and 495 adults (55.7%) showed the highest abundance and distribution in the county calling for more studies on their population, ecology, behavior and probable roles as vectors of various diseases.

### INTRODUCTION

Approximately 3500 species of mosquitoes were reported from different parts of the world of which *Culex*, *Aedes*, *Anopheles*, and *Ochlerotatus* are very important genera (Service, 2012). *Culex* and *Anopheles* showed the most important public health significance because of their capability to transmit diseases such as malaria, filariasis

and arboviral diseases to humans (Kumar & Hwang, 2006). Considering the incidence rate, mortality and morbidity, malaria is still the number one killer in more than 100 developing countries in tropical and subtropical areas. In 2015, globally 214 million positive cases (range: 149–303 million) of malaria with 429,000 deaths (including 303 000 children under 5 years old) occurred. Most of the cases were reported

from Africa (90.0%), followed by South-East Asia (7.0%) and Eastern Mediterranean Region (2.0%) (WHO, 2015). Significant progress has been made in malaria elimination in Iran, however it is still endemic in Iran and few indigenous cases of malaria are reported from Sistan and Baluchistan, Kerman and Hormozgan Provinces. Sporadic local transmission as well as imported cases of malaria are reported from 19 other provinces of the country including the Northern Province of Mazandaran (Salehi *et al.*, 2014). Out of 30 *Anopheles* species identified in Iran, 8 species are considered as vectors of malaria including *An. culicifacies*, *An. d'thali*, *An. sacharovi*, *An. maculipennis*, *An. fluviatilis*, *An. stephensi*, *An. pulcherrimus* and *An. superpictus* (Sedaghat & Harbach, 2005, Djadid *et al.*, 2009, Azari-Hamidian, 2007).

In the recent years, concerns have been raised over the rapid spread of dengue fever in various countries including Pakistan (Brady *et al.*, 2012). Imported cases of dengue fever (Chinikar *et al.*, 2013), West Nile virus and *Dirofilaria immitis* were reported from Iran (Chinikar *et al.*, 2011, Azari-Hamidian *et al.*, 2009).

The morphological key of Iranian *Anopheles* was developed by Shahgudian (1969). Zaim *et al.* (1984, 1985, 1986) who conducted a comprehensive research on Iranian mosquitoes, followed by a checklist of Iranian mosquitoes published in 2009 (Azari-Hamidian & Harbach, 2009). Faunistic and ecological studies have also been undertaken on Culicidae in various parts of Iran including Guilan (Azari Hamidian *et al.*, 2001, 2003). Zanzan (Ghavami & Ladoni, 2005), Chabahar (Moosa-Kazemi *et al.*, 2009), Qom (Saghafipour *et al.*, 2012). According to these studies 7 genera, 64 species and 3 subspecies of Culicidae have been identified from Iran (Azari-Hamidian, 2007).

There are few reports of Culicidae in Mazandaran, North of Iran. Recently, Nikookar *et al.* (2010, 2015a,b) studied the fauna, seasonal activity, larval habitat characteristics and biodiversity of Culicidae in Neka County. In the wake of the rising risk of emerging and reemerging vector borne

diseases especially Zika and dengue and despite the number of studies performed on Culicidae in Iran, still a lot needs to be learnt about the biology and ecology of larvae and adults of Culicidae for the implementation of proper control measures in Iran and particularly in Mazandaran. No entomological studies had been done in Noor County so far and because of the suitability of the environment for mosquitoes to thrive and the risk of vector borne diseases in the area, this research was designed to identify mosquito fauna and their seasonal activity in Noor northern Iran.

## MATERIALS AND METHOD

The research was a descriptive cross-sectional entomological faunistic study. In each three rural regions, nine villages and three locations from outskirts of the city were visited monthly from mid-August 2012 until November 2013 and Culicidae larvae, pupae and adults were collected.

Noor is located in northern Iran in Mazandaran Province covering 2,675 km<sup>2</sup>. It consists of two mountainous and plain areas between the longitude of 51° 26' East and latitude of 36° 47' North. The studied villages in Noor were: Abasa, Enarjar, Selyakati, Nematabad, Moghandeh, Abdalkeh, Shirkola, Rozak, Guilandeh and three urban outskirts were Noor, Rouyan and Chamestan.

### Larval collection

Larvae collected by standard 350cc dipper from different types of natural and artificial breeding places. Buckets were used for sampling larvae from wells, and dropper was used for small larval habitats. The collected larvae were kept in lactophenol solution for a week for larvae to become transparent in preparation to be identified.

### Adult collection

Adult mosquitoes were collected by total catch and hand catch using aspirators. The specimens were put in paper cups and by providing the necessary moisture, they were transferred to Medical Entomology

Laboratory at Sari Health Faculty for identification.

### Identification

The collected specimens were identified to species under a microscope (larvae) and stereomicroscope (adults) using the keys of Shahgudian (1960), Zaim & Cranston (1986) and Azari-Hamidian and Harbach (2009).

## RESULTS

A total of 844 Culicidae larvae and 1 484 adult mosquitoes were collected in this study. Of these, 179 and 595 larvae and adults belonged to Anophelinae and 665 and 889 larvae and adults belonged to Culicinae. In April, May, October and November, no Anopheline larvae

and adults were collected while Culicine larvae and adult specimens (*Cx. pipiens*, *Cx. theileri* and *Cx. mimeticus*) were collected in May. The larvae and adults of *An. hyrcanus* and *Cx. pipiens* showed peak activity in July and August but *An. hyrcanus* larvae peaked in August (Table 1).

Of 179 collected Anopheline larvae, 83 were *An. maculipennis s.l.* and 96 were *An. hyrcanus*. Also out of 665 caught Culicinae larvae, 501 were *Cx. pipiens*, 108 *Cx. mimeticus* and 56 *Cx. theileri* (Table 2).

Using hand catch, 112 *An. hyrcanus*, 88 *An. maculipennis s.l.*, 103 *Cx. pipiens*, 54 *Cx. mimeticus* and 65 *Cx. theileri* were collected. Using total catch, 259 *An. hyrcanus* and 136 *An. maculipennis s.l.*, 392 *Cx. pipiens*, 102 *Cx. mimeticus* and 173 *Cx. theileri* were collected (Table 2).

Table 1. Temporal distribution of mosquito species collected from the study areas in Noor County, Mazandaran Province, in 2012-2013

Month	Species									
	<i>An. maculipennis s.l.</i>		<i>An. hyrcanus</i>		<i>Cx. theileri</i>		<i>Cx. mimeticus</i>		<i>Cx. pipiens</i>	
	larva	adult	larva	adult	larva	adult	larva	adult	larva	adult
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	1	5	2	8	17	12
June	3	7	5	38	2	35	14	39	34	61
July	33	62	32	98	6	38	18	67	130	121
August	36	84	50	95	17	41	26	57	144	127
September	10	59	7	94	21	20	29	34	137	98
October	1	12	2	46	8	11	17	22	33	52
November	0	0	0	0	1	6	2	11	4	19
December	0	0	0	0	0	0	0	0	2	0
Total	83	224	96	371	56	156	108	238	501	495

Table 2. Distribution of mosquitoes collected from the study areas in Noor County, Mazandaran Province, by collection methods in 2012-2013

Catching method	Species									
	<i>An. maculipennis s.l.</i>		<i>An. hyrcanus</i>		<i>Cx. theileri</i>		<i>Cx. mimeticus</i>		<i>Cx. pipiens</i>	
	larva	adult	larva	adult	larva	adult	larva	adult	larva	adult
Hand catch	0	80	0	112	0	54	0	65	0	103
Total catch	0	136	0	259	0	102	0	173	0	392
Dipping	83	0	96	0	56	0	108	0	501	0
Total	83	224	96	371	56	156	108	238	501	495

*Anopheles hyrcanus* larvae were the most frequent (22.92% of total specimens) from Noor urban outskirts and the lowest (2.19%) in Rozak rural area. *An. maculipennis s.l.* larvae were of the highest frequency (30.12%) in Rozak and the lowest (4.82%) from rural Nematabad, Abasa, Moghandeh and Chamestan margin. The maximum (13.8%) *Cx. pipiens* larvae were collected from Noor outskirts and the minimum (3.59%) from Moghandeh rural district. The highest (16.7%) *Cx. mimeticus* larvae were from Selyakati margin and the lowest (3.70%) from Chamestan outskirts. The maximum (16.07%) *Cx. theileri* were from Noor and Enarjar outskirts and the minimum (1.79%) from Chamestan outskirts (Table 3).

The highest (18.1%) *An. hyrcanus* adults collected were from Noor outskirts and the lowest (6.19%) from Rozak rural district. The maximum (16.5%) *An. maculipennis s.l.* adults collected were from Rouyan margin and the minimum (4.02%) from Guilandeh rural district. The highest (15.8%) *Cx. pipiens* were from Noor margins and the lowest (5.45%) from Abdalkeh. The highest (16.4%) *Cx. mimeticus* adults collected were from Selyakati outskirts and the lowest (4.62%) from Nematabad rural district. The maximum (12.18%) *Cx. theileri* adults caught were from Enarjar and Nematabad rural district and the minimum (5.64%) from Chamestan outskirts (Table 3).

## DISCUSSION

Five species belonging to two genera were collected and identified from nine rural and three geographical districts in the outskirts of Noor County. These species have been reported in recent years from Mazandaran Province (Nikookar *et al.*, 2015b, 2016) except *Cx. theileri*.

*Anopheles hyrcanus* was the most frequent *Anopheles* species in the present study. This species was reported as a wild species by Zahar (1990) a finding that is not supported by the current and other studies (Azari-Hamidian *et al.*, 2003). *Anopheles hyrcanus* were found from indoor resting

places in particular from barns with 88.24% in Guilan province (Azari-Hamidian *et al.*, 2003) and also in very low frequency from toilets and residential places (Azari-Hamidian *et al.*, 2003). This is in agreement with the results of the present study.

*Anopheles hyrcanus* was found for the first time from the southern shores of the Caspian Sea (Djadid *et al.*, 2009). It is listed as a member of the *Hyrceanus* group (Azari-Hamidian 2007) and also as the most prevalent species in northwestern Iran (Vatandoost *et al.*, 2005, Yaghoobi-Ershadi *et al.*, 2001). In fact, it could possibly be important that they did not distinguish members of *Hyrceanus* group from each other. That is why some researchers believe that the species is less abundant than other members of the group. (Dow, 1953, Minar, 1974, Azari-Hamidian *et al.*, 2001), however, the present study could not reproduce these observations.

Larvae and adult *An. maculipennis s.l.* have been collected in the county with different ratios. The maximum number of this species caught was by total catch method in animal places. This species has been reported in animal places up to 93.6% and its larvae were collected from river bed pools, rain pools, and rice fields from Guilan (Azari-Hamidian *et al.*, 2003, Azari-Hamidian, 2011). Nikookar *et al.* (2015b) reported larvae of this species up to 0.54% in three villages of Neka County. The members of this species complex had the highest occurrence in livestock barn (Djadid *et al.*, 2009) which is in accord with our study.

*Anopheles maculipennis s.l.* is a malaria vector in central Iran plateau and North of the country and some of its species have caused reemerging malaria in Guilan, West Azerbaijan and Ardebil (Edrissian, 2003). The results of this study showed that barns of domestic animals are the best resting place for the members of *An. maculipennis* group, a finding supported by the results of a study in Guilan Province, North of Iran (Azari-Hamidian *et al.*, 2003). It should be noted that, as Dow indicated, the daily resting places of mosquitoes are not necessarily their blood sucking spot (Dow, 1953). Therefore, further studies are needed to associate the resting

Table 3. Spatial distribution of mosquitoes collected from the study areas in Noor County, Mazandaran province, in 2012-2013

Collection place	Species																		
	<i>An. hyrcanus</i>			<i>An. maculipennis s.l.</i>			<i>Cx. pipiens</i>			<i>Cx. mimeticus</i>			<i>Cx. theileri</i>						
	larva	adult	%	larva	adult	%	larva	adult	%	larva	adult	%	larva	adult	%				
Abasa	14	33	8.9	4	4.83	14	6.25	51	10.17	44	8.89	7	6.48	22	9.25	5	8.93	16	10.26
Enarjar	12	25	6.74	5	6.02	19	8.48	39	7.78	32	6.46	8	7.41	19	7.98	9	16.07	19	12.18
Selyakati	8	36	9.7	5	6.02	16	7.14	47	9.38	37	7.47	18	16.7	39	16.4	6	10.71	17	10.9
Nematabad	7	21	5.66	4	4.82	17	7.59	45	8.98	49	9.9	16	14.81	11	4.62	5	8.93	19	12.18
Moghandeh	4	21	5.66	4	4.82	11	4.91	18	3.59	28	5.66	13	12	18	7.57	2	3.57	7	4.49
Abdaldeh	4	32	8.63	5	6.02	24	10.7	32	6.39	27	5.45	8	7.41	16	6.72	6	10.71	12	7.69
Shirkola	3	34	9.16	7	8.44	18	8.03	45	8.98	36	7.27	9	8.34	27	11.34	4	7.15	11	7.05
Rozak	4	28	7.55	25	30.12	15	6.7	39	7.78	29	5.86	7	6.48	16	6.72	2	3.57	9	5.77
Guilandeh	2	17	3.77	8	9.64	9	4.02	32	6.39	31	6.26	6	5.56	15	6.3	3	5.36	14	8.97
<sup>a</sup> U.O. Noor	22	67	18.1	7	8.43	25	11.2	69	13.8	78	15.8	7	6.48	16	6.72	9	16.07	17	10.9
U.O. Rouyan	9	38	10.2	5	6.02	37	16.5	48	9.58	63	12.7	5	4.63	18	7.56	4	7.14	11	7.05
U.O. Chamestan	7	22	5.93	4	4.82	19	8.48	36	7.18	41	8.28	4	3.7	21	8.82	1	1.79	4	2.56

<sup>a</sup>U. O. : Urban Outskärt

and feeding habits of the members of *An. maculipennis* group in the region.

Three species of *Culex* were found in this study. *Culex pipiens* was one of the most common Culicine mosquitoes collected in Noor County. These observations are in accord with other studies (Azari-Hamidian *et al.*, 2001, Nikookar *et al.*, 2010, 2015b, Khoshdel-Nezamiha *et al.*, 2014). This species showed the highest monthly activity from July to September in the current study. Nikookar *et al.* (2010) collected *Cx. pipiens* larvae from tree trunk hole accompanying with the species *Cs. annulata*, *Oc. geniculatus*, *Oc. echinus* and *An. plumbeus* with peak activity in mid-July. In other similar study, abundance of this species was reported in three villages from Neka County up to 63.99% (Nikookar *et al.*, 2015b). This species was found up to 12.05% of the total mosquitoes in Turkey (Gunduz *et al.*, 2009).

*Culex mimeticus* was the second abundant species of the genus *Culex* in our investigation. The results are almost the same as those of previous studies in Mazandaran and West Azerbaijan Provinces (Khoshdel-Nezamiha *et al.*, 2014, Nikookar *et al.*, 2015b). This species was collected only as larvae by Saghafipour *et al.* (2012) and adult by Azari-Hamidian *et al.* (2003), whereas both larvae and adults were collected in the present study. The global distribution of this species includes Southern Palaearctic and Oriental areas (Knight & Stone, 1977), and has not been confirmed as a specific vector of diseases.

*Culex theileri* was collected with the lowest frequency in our study, whereas in Turkey (Alkan & Aldemir, 2010) and northern and central parts of Iran, it is the most dominant species, except for the low-lying areas bordering the Caspian Sea and in urban areas where *Cx. pipiens* is the most prevalent species (Khoshdel-Nezamiha *et al.*, 2014). This species is distributed in parts of the world (Knight & Stone, 1977) and almost all of Iran (Dow, 1953, Moosa-Kazemi *et al.*, 2009, Khoshdel-Nezamiha *et al.*, 2014).

The existence of *An. hyrcanus* and *Cx. pipiens* in the Noor County as predominant species is significant because both of these species are vectors of diseases. Therefore

these findings indicate the need for more studies on vectors and the diseases they may transmit in the County.

In conclusion, the results of the present study showed that *Cx. pipiens* and *Cx. theileri* had the highest and the lowest abundance and distribution in Noor County respectively. Ecology of *Cx. pipiens*, the most frequent species which is conceivably involved in the transmission of many pathogens to humans and domestic animals, should be comprehensively studied in the future.

## DISCLOSURE

The authors declare that they have no conflict of interests.

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