

## Original Articles

# Species Composition and Diversity of Mosquitoes in Neka County, Mazandaran Province, Northern Iran

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### Abstract

**Background:** Regarding to the significant of the possibility of the malaria epidemic and nuisance of mosquitoes during the active season, the fauna and some ecological activities of mosquitoes in related to tree holes were investigated from April to December 2009 in Neka county of Mazandaran Province, northern Iran.

**Methods:** Larval collection was carried out from natural, artificial breeding places, and tree holes inside the forest in Neka County, Mazandaran Province in 2009. In addition, human bait net trap collection was conducted using suction tube several times during this investigation.

**Results:** Four genera and five species were found in tree holes. *Anopheles plumbeus*, *Culiseta annulata*, *Culex pipiens*, and *Ochlerotatus geniculatus* were collected by larval collection whereas, *Ochlerotatus pulcritarsis* was found by adult collection. Overall *Cx. pipiens* 44.6%, *Oc. geniculatus* 32.6%, *An. plumbeus* 22.5%, and *Cs. annulata* 0.3% were collected by larval collection. During the bait net collection five specie were identified including: *Oc. geniculatus* 55.87%, *Oc. echinus* 1.33%, *Oc. pulcritarsis* 8.8%, *Cx. pipiens* 33.8%, and *An. plumbeus* 0.2%. *Cs. annulata* larvae was detected for the first time with a low abundance in tree cavity.

**Conclusion:** Tree holes were found the main habitat for the species of *Oc. geniculatus*. The species of *Cs. annulata* was found in tree holes

**Keywords:** Mosquito, fauna, tree holes, diversity, Iran

### Introduction

The family of Culicidae (Diptera) divided in two subfamily, 11 tribes, 113 genera, and 3526 species in the world fauna (Harbach 2007). Culicidae mosquitoes are the most public health important vectors of arthropods due to malaria transmission and vectors of various types of filariasis, and arboviruses (Horsfall 1955, Smith 1973, Zaim et al. 1984, Service 1993). Arboviral viruses such as West Nile were reported previously in Iran (Saidi et al. 1976). Dog heart worm parasite (Saidi et al. 1976, Mobedi et al. 1990) and *Dirofilaria repens* (Sadighian 1969, Siavashi and Massoud 1995) also were reported previously in country. The dog heart worm, *Dirofilaria immitis* transmitted by tree

holes mosquitoes and have been reported from Spain (Bargues et al. 2006). *An. plumbeus* has been reported the vector of *Plasmodium falciparum* in Germany (Kruger et al. 2001).

Although, larval breeding places and blood feeding behavior of malaria vectors have been studied previously, but in relation to species composition, and diversity of tree holes mosquitoes there are a few data. Preliminary studies in relation to identification of Culicidae mosquitoes of Iran and its role in disease transmission such as malaria have been reported by Macan and Gutsevich (Gutsevich 1943, Macan 1950). There is scattered information due to Culicidae mosquitoes and re-

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ported by Dow in 1953, Minar in 1974, and 1981 (Dow 1953, Minar 1974, 1981). Zaim et al. reported the fauna of *Aedes* mosquitoes in Iran (1984). Checklist of the culicinae mosquitoes of Iran was reported by Zaim and Cranston in 1986 (Zaim and Cranston 1986). Fauna and ecological characteristics of Culicidae mosquitoes in Isfahan, Kurdistan, Sistan and Baluchistan and Hormozgan Provinces have reported previously (Mousakazemi et al. 2000, Moosa-Kazemi et al. 2005, 2009, and 2010). The fauna and ecological study of Culicidae mosquitoes in Guilan Province was reported by Azari-Hamidian in 2002 (Azari-Hamidian et al. 2002a). Azari-Hamidian reported the tree holes culicidae mosquitoes in Guilan Province previously (Azari-Hamidian 2003). Some mosquitoes species such as *An. plumbeus*, *Oc. echinus*, *Oc. geniculatus*, *Orthopodomyia*, *Oc. pulcritarsis*, have been reported as tree holes mosquitoes in Greece (Silankok et al. 1937). The recent species also were reported in France (William et al. 1986) and Azerbaijan (Nagiyev 1963). Investigation on species composition and diversity of mosquitoes' is important in different aspects of vector control programmers. This information use to decrease of vector density then the hosts and vectors contacts, and biting nuisance.

The present study was focused on some ecological aspects of the mosquitoes in relation to tree holes, such as the species composition, and diversity of mosquitoes in Neka County, northern Iran.

## Materials and Methods

The study was carried out from April to December 2009. Three fixed sites (Darvishan, Chalmardi, and Khairabad), and six variable sites (Golbestan, Elmiyeh, Baghearab, Nimchah, Chalehpol, and Khorshid) were selected randomly with regard to existing facilities in Mazandaran Province (36°39'N, 53°17'E) (Fig. 1). Maximum and minimum temperatures were 27.6 and 9.1°C in the July and December,

respectively. The maximum annual rainfall has been reported 204.6 mm in October, and the least 0.1 mm in July 2009. The type of forest vegetation has been found *Quercus castanifolia*, *Alnus subcerdata*, *Carpinus betulus*, *Zelkova carpinifolia*, and *Mespilus sp* (Natural Resources of Neka County 2009).

Sampling in larval habitats was performed by standard dipper biweekly, and adult mosquitoes were collected by one human bait net trap using suction tube monthly. The mosquito larvae were collected in different oviposition sites such as discarded tires, tree and rock holes, irrigation channels, rice fields, ponds, animal footprint, and marshes inside forest. Some mosquito larvae were collected from 18 tree holes using dropper. These 18 tree holes were also evaluated two times monthly. During each of the evaluations, pupa were collected and placed in Bucket dishes lined with netting and transferred to the laboratory. Hatched pupa were reared to adult, identified by using the keys of Shahgudian (1960), Zaim and Cranston (1986), Azari-Hamidian and Harbach (2009).

Human bait net collections were conducted monthly randomly nears the tree holes inside the forest. The mosquitoes were transferred to cups container, was kept closed and then collected with forceps and placed in Petri-dishes lined with moist cotton. The samples were sent to the Laboratory of Entomology, School of Public Health, and Tehran University of Medical Sciences.

The mosquito larvae were preserved in lactophenol and the microscopic slides were prepared using de Faure's medium after one week (WHO 1963). Adult mosquito samples were killed by chloroform and mounted by special pines. The samples were identified using the keys mentioned above. The weather condition such as temperature, humidity and altitude were recorded on the standard forms. The mosquito name abbreviations were cited based on Reinert (Reinert 2009). The data were analyzed using SPSS ver. 11.5.

## Results

A total of 3047 adult mosquitoes were collected. Out of 1547 adult mosquitoes were reared from pupae and the rest was collected by bait net trap. A total of 960 *Cx. pipiens* (62.06%), 568 *Oc. geniculatus* (36.71%), and 19 *An. plumbeus* (1.23%) were reared to maturity during 18 sampling occasions from different larval habitats (Table 1). Totally 1500 mosquitoes were collected during the human bait net trap collection during 24 h. The most prevalent species was *Oc. geniculatus*, a total of 838 (55.87%), followed by *Cx. pipiens* 507

(33.8%), *Oc. pulcritarsis* 132 (8.8%), *Oc. echinus* 20 (1.33%), and *An. plumbeus*, 3 (0.2%) (Table 2). The peaks of blood feeding activity of *Oc. echinus*, *Oc. geniculatus*, *Oc. pulcritarsis*, and *Cx. pipiens* were the same and found as 11.00-12.00 AM, and 16.00-17.00 PM. (Table 2). A total of 1482 mosquito larvae collected during the study comprising *Cx. pipiens* 661 (44.6%), *Oc. geniculatus* 483 (32.6%), *An. plumbeus* 333 (22.5%), and *Cs. annulata* 5 (0.3%) (Table 3). It should be noted that these species have been collected only in trees holes in this area.

**Table 1.** Frequency of the adult mosquitoes reared from pupae in Neka County, Mazandaran Province, 2009

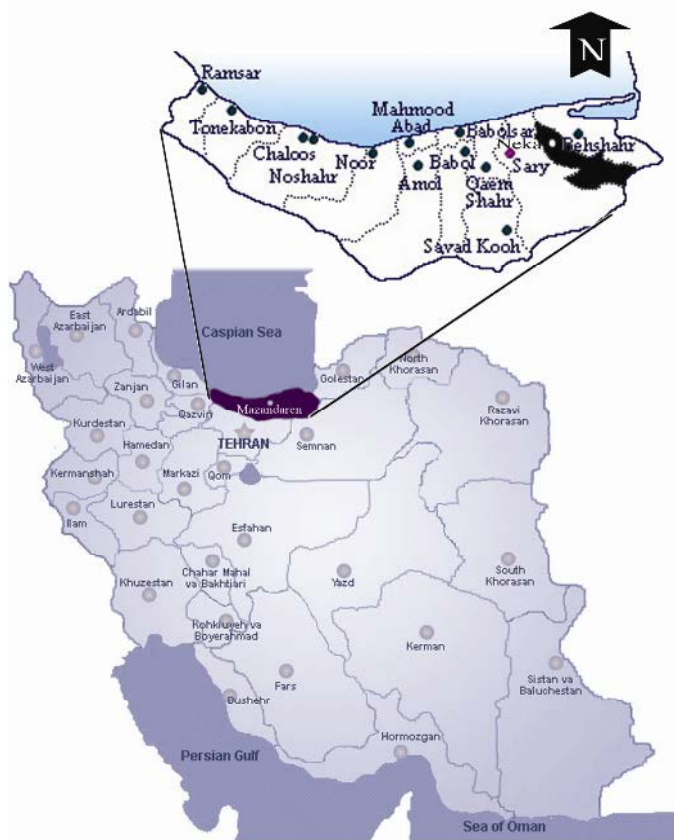
Species	Apr.		May		Jun.		Jul.		Aug.		Sep.		Oct.		Nov.		Dec.		Total		
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
<i>An. plumbeus</i>	0	0	0	0	1	5.26	2	10.52	0	0	6	31.57	5	26.31	4	21.05	1	5.26	19	100	
<i>Cx. pipiens</i>	28	2.91	80	8.33	120	9.6	88	9.16	0	0	300	31.25	154	16.04	101	10.52	89	9.27	960	100	
<i>Oc. geniculatus</i>	5	0.88	12	2.17	20	3.52	80	14.08	0	0	200	35.21	96	16.90	79	13.90	76	13.38	568	100	
<b>Total</b>																				1547	100

**Table 2.** Abundance of adult mosquitoes collected by human bait net trap in Neka County, Mazandaran Province, 2009

Collection hours	Number of Species				
	<i>An. plumbeus</i>	<i>Cx. pipiens</i>	<i>Oc. geniculatus</i>	<i>Oc. echinus</i>	<i>Oc. pulcritarsis</i>
05-06 AM	0	0	0	0	0
06-07 AM	0	0	0	0	0
07-08 AM	0	0	0	0	0
08-09 AM	0	0	0	0	0
09-10 AM	0	4	8	0	0
10-11 AM	0	8	20	1	4
11-12 AM	0	36	200	5	20
12-13 PM	0	16	32	2	3
13-14 PM	0	9	20	1	2
14-15 PM	0	2	3	0	1
15-16 PM	0	4	5	1	3
16-17 PM	0	380	430	7	60
17-18 PM	0	38	100	2	30
18-19 PM	0	10	20	1	9
19-20 PM	0	0	0	0	0
20-21 PM	0	0	0	0	0
21-22 PM	1	0	0	0	0
22-23 PM	2	0	0	0	0
23-24 PM	0	0	0	0	0
00-01 AM	0	0	0	0	0
01-02 AM	0	0	0	0	0
02-03 AM	0	0	0	0	0
03-04AM	0	0	0	0	0
04-05AM	0	0	0	0	0
<b>Total</b>	3 (0.2%)	507 (33.8%)	838 (55.87%)	20 (1.33%)	132 (8.8%)

**Table 3.** Density of larval mosquitoes per 10 dips collected from tree holes in Neka County, Mazandaran Province, 2009.

Species	Apr.			May			Jun.			Jul.			Aug.			Sep.			Oct.			Nov.			Dec.			Total	
	n	D	%	n	D	%	n	D	%	n	D	%	n	D	%	n	D	%	n	D	%	n	D	%	n	D	%	Total	%
<i>An. plumbeus</i>	3	1.5	0.9	6	3	1.8	28	14	8/4	36	18	10.81	0	0	0	100	50	30.03	86	43	25.82	50	25	15.01	24	12	7.20	333	22/50
<i>Cs. annulata</i>	0	0	0	5	2.5	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0/30
<i>Cx. pipiens</i>	11	5.5	1.65	30	15	4.50	100	50	15.01	130	65	19.51	0	0	0	150	75	22.52	120	160	18.01	80	40	12.01	40	20	6	661	44/60
<i>Oc. geniculatus</i>	1	0.5	0.2	26	13	5.38	66	33	13.66	82	41	16.97	0	0	0	124	62	25.67	106	53	21.94	50	25	10.35	28	14	5.79	483	32/60
<b>Total</b>																												1482	100



**Fig. 1.** Map of Iran, highlighting the position of Neka County in Mazandaran Province

## Discussion

In the present investigation four genera and five species were found in tree holes. Several species of tree holes mosquito have medical importance and bite only during the daytime (diurnal).

*Ochlerotatus echinus* has been distributed in the Mediterranean region, North Africa, Asia and southern Europe, Greece, Algeria, Morocco, Spain and France (Knight and Stone 1977). The larvae of this species collected for the first time in Sari of Mazandaran Province by Janbaksh in 1955 (Zaim et al. 1984). The larvae have been found from Rezvanshahr, Shaft, Fuman and Masal Counties in Guilan Province (Azari-Hamidian et al. 2002b). In our study, the species was collected from tree holes in the Neka County. There is little data about the ecology of this species in Iran (Azari-Hamidian 2003). *Ochlerotatus geniculatus* was found with 55.86 of the total adult mosquito collection. This species was distributed in the Europe, North Africa, South-East Asia (Knight and Stone 1977). Gutsevich (1943) reported this species for the first time in Iran. This species was reported from Ardebil, Golestan, Mazandaran, Guilan Provinces (Gutsevich 1943, Zaim 1987, Azari-Hamidian et al. 2002b, Azari-Hamidian et al. 2009). This species feeding on mammalian animals, and has exophagic behavior (Horsfall 1955). Yellow fever and eastern equine encephalitis viruses have been transmitted by this species in the lab (Yates et al. 1979, Horsfall 1955). In our study a total of 483 (32.6%) of larval collection, 568 (36.71%) from pupa mature, and 838 (55.87%) were collected by human bait trap respectively.

*Ochlerotatus pulcritarsis* has been reported from Mazandaran Province previously (Zaim 1987, Minar 1981, Zaim et al. 1984). During the study, a total of 132 (8.8 %) was collected by human bait trap, whereas no larvae was found among breeding places. The peak of the blood activity of this species was found

11.00–12.00 A.M and 16.00–17.00 P.M. Although other Aedini have reported in Iran, such as *Oc. flavescens* from West Azerbaijan (Zaim 1987, Zaim et al. 1984), *Oc. vittatus*, *Oc. detritus*, and *Oc. leucomelas* from Hormozgan and Sistan and Baluchestan Provinces (Zaim 1987, Zaim et al. 1984), *Ae. aegypti* from Bushehr Province (Dow 1953), *Oc. caspius* from Isfahan (Mousakazemi et al. 2000, Moosa-Kazemi et al. 2010) Guilan (Azari-Hamidian et al. 2002) but not found in this area.

*Culiseta annulata* has been distributed in the western region of Palearctic (Knight and Stone 1977). This species was reported from Iran by Gutsevich in 1943 (Gutsevich et al. 1970), and by Minar in 1981 (Minar 1981). Azari-Hamidian reported from Rudbar, Shaft, Fouman, Langeroud and Masal cities in northern part of Iran (Azari-Hamidian 2003b, Azari-Hamidian 2005), however without association with *An. plumbeus*. In our study this species was collected in association with *An. plumbeus*, *Cx. pipiens*, and *Oc. geniculatus*. In our study it was found during the larval collection from tree holes. This is the first report of the existence of this species in tree holes in Iran. The species of *Cs. subochrea* was reported in Mazandaran Province whereas, *Cs. annulata* not reported by Zaim (1987). These two recent species are very similar in larval stage; however they are distinguishable as adult. The taxonomy and distribution of three close species, *Cs. alaskaensis*, *Cs. annulata*, and *Cs. subochrea* in Iran should be considered in future studies.

In our study, one species of the genus *Anopheles* was identified based on the morphological characters of larvae and adult.

Shagudian (1960) mentioned some characters to distinguish of the larvae. Lateral hairs on abdominal segments IV–VI of this species distinctly feathered and frontal hairs are simple. *Anopheles plumbeus* is reliably distinguishable from other species in adult stage. Fore margin



of wing is uniformly dark, wing with distinct dark spots, upper surface of thorax dark at the side with a broad lighter stripe down the middle, white frontal tuft present. Shagudian (1960) mentioned, proboscis of female uniformly brown and last segment of female palpus more than half as long as the penultimate. The last character mentioned to distinguish the adult of *An. plumbeus* from those of *An. claviger*.

*Anopheles plumbeus* represented 22.5% of larval collection, 1.23% from rearing to mature, and 0.2% from human bait net trap collection. Horsfall in 1955 reported the association of this species with *Ae. aegypti*, *Cs. annulata*, *Cx. pipiens*, *Oc. geniculatus*, *Oc. echinus*, and *Orthopodomyia pulcripalpis*. It should be mentioned *Or. pulcripalpis* has not been reported in Iran but *Ae. aegypti* has been reported previously (Azari-Hamidian et al. 2007).

*Culex pipiens* distributed in Europe, the tropical and subtropical regions of Asia, Africa the middle part of North America, Southern America, and Australia (Vinogradova 2000). This species is distributed in the most part of Iran (Zaim et al. 1985). Distribution of this species has close relationship with economic activities and development of new territories. The human activity on natural environment with a change in the land and underground water resources can be affect the abundance of this species (Vinogradova 2000). *Culex pipiens* has at least three complex system of environmental compliance. *Culex quinquefasciatus* has been distributed in the tropical area with the wide range of hosts. *Culex pipiens* has been distributed in the moderate area with the limited range of birds nest maker (Mousakazemi et al. 2000). *Culex pipiens* form *molestus* is autogenous, and the first laying eggs perform without a blood feeding (Vinogradova 2000). *Culex pipiens* form *molestus*, *Cx. quinquefasciatus* and possibly *Cx. pipiens pallens* need to the small space for mating whereas, a large space need to mate for anautogenous *Cx. pipiens pipiens* (Vinogradova 2000). In our research *Cx. pipiens* was found in tree

holes. Azari-Hamidian et al. (2001, 2002a) reported it as one of the predominant species in Guilan Province, northern Iran, and in central part of Iran (Mousakazemi et al. 2000). This species was found as 12.05% of the total mosquito in Turkey (Gunduz et al. 2009). In our research monthly activity of *Cx. pipiens* larvae began in the end of May, and was increased in the beginning of July and decreased slowly in the mid of summer. The main peak of the activity of adults was determined in mid of July. The monthly activity of larvae near Moscow City was started from late April to May when the weather conditions was changed, and the first group of eggs and larvae was appeared in mid-May when the average temperature between 10 and 15 degrees centigrade and was matured in late May to early June (Vinogradova 2000). Agree with our research, Mousakazemi et al. reported that the *Cx. pipiens* larval activity was begun from late May and a peak in early July (Mousakazemi et al. 2000). The second peak reported in the late July. The seasonal activity was decreased in early October. Activity of adult was reported in the late May and reached to peak in the mid of July and then decreased to zero in October (Mousakazemi et al. 2000). In our research peak blood feeding of *Cx. pipiens* was found 11.00–12.00 M and 16.00–17.00 PM. Vinogradova (2000) reported that this species can change shelter in urban areas.

Azari Hamidian reported that 66.3% of the total of *Cx. pipiens* collected in indoor places. Stable, toilet, and chicken compliments with cement, plaster, straw and wooden walls were reported the main resting places. He mentioned 33.7% of this species was collected from outdoor places such as gramineous plants, inside the wells, discarded tire (Azari-Hamidian et al. 2003a). It should be mentioned that the resting behavior depends on the climate and human economic activities which result in more or less to exophily and endophily behavior. *Culex pipiens* was found in Neka,

with total of 661 (44.6%) larvae from tree holes and 507 (33.8%) from human bait net trap.

In conclusion, there are several plant species such as *Quercus castanifolia*, *Alnus subcordata*, *Carpinus betulus*, *Zelcova carpinifolia*, and *Mespilus* species (Natura Resources of Neka County 2009). However, the important and interesting thing in our study is that the larvae of *Oc. geniculatus* was found only in the *Q. castanifolia* species, whereas *An.plumbeus*, *Cs. anulata* and *Cx. pipiens* were found in the *Q. castanifolia*, pools, disposal tire, and other larval habitat. In this study, the minimum and maximum temperature of water inside the cavity were 14°C and 16° C respectively. The range of pH was 4–5.5. Due to present of shade and wind near the tree holes, the range of temperature remind constant as 14–16° C. Rice fields as breeding places for mosquitoes have been reported in the areas of Kenya (Mwangangi et al. 2010), but in our study despite the great effort, except of *Cx. pipiens*, the other species were not found within rice fields. Larval habitat epiphytic algae, aquatic and semi aquatic species, electric conductivity (EC), dissolved oxygen, turbidity, disintegrated organic and inorganic material, pesticide toxic, and predators should be considered in future studies.

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