

The fauna and ecology of mosquito larvae (Diptera: Culicidae) in western Iran

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Abstract: Ecological data are important in integrated vector management. There are few published documents about the ecology of mosquitoes (Diptera: Culicidae) in western Iran. To study the fauna and ecology of the mosquito larvae of Kurdistan Province, the samples were collected by the dipping method during June–October 2005 and June–August 2006. Larval habitat characteristics were recorded according to habitat type, water conditions (stagnant or running, clear or turbid), water temperature, vegetation (with or without vegetation), and sunlight exposure (full or partial sunlight or shaded). A total of 2096 third- and fourth-instar larvae were collected. Four genera and 11 species were identified: *Anopheles claviger* (1%), *An. maculipennis* sensu lato (s.l.) (17.4%), *An. superpictus* (57.7%), *Culex hortensis* (1.5%), *Cx. mimeticus* (2.7%), *Cx. perexiguus* (0.2%), *Cx. pipiens* (0.8%), *Cx. theileri* (12.2%), *Culiseta longiareolata* (6.1%), *Cs. subochrea* (0.3%), and *Ochlerotatus caspius* s.l. (0.1%). *Anopheles superpictus*, *An. maculipennis* s.l., and *Cx. theileri* were the most abundant and widely distributed species. All larvae were collected from natural habitats (river edges or ground pools). There was no significant difference among the mean water temperatures (17.7–26 °C) of the habitats of different species ($P = 0.098$). The larval habitat characteristics, association occasions, and percentages of each species are discussed.

Key words: *Anopheles*, *Culex*, *Culiseta*, *Ochlerotatus*, Culicidae, mosquito, larval habitat, Kurdistan Province, Iran

1. Introduction

According to the most recent classification of mosquitoes (Diptera: Culicidae), the family includes 2 subfamilies, 11 tribes, 111 genera (43 genera based on the earlier classification), and 3528 species in the world fauna; the genus *Anopheles* Meigen includes 7 subgenera and at least 465 species (Harbach, 2007).

The checklist of the mosquitoes of Iran includes 7 genera and 64 species (Azari-Hamidian, 2007a). Oshaghi et al. (2008) recently identified *Anopheles superpictus* Grassi as a complex of 3 genotypes (X, Y, and Z), and Djadid et al. (2009) proposed a new species of the *Hyrchanus* group based on DNA sequence data. Naddaf et al. (2010) reported *An. fluviatilis* James species U from Fars Province and were in doubt about the occurrence of the species (form) V in Iran (Azari-Hamidian, 2007a). Mehravaran et al. (2011) also recorded species U in southeastern Iran.

Sindbis and West Nile viruses have been reported in Iran (Naficy and Saidi, 1970; Saidi, 1974; Saidi et al., 1976; Sharifi et al., 2010; Ahmadnejad et al., 2011; Chinikar et al., 2011; Fereidouni et al., 2011). Chinikar et al. (2010) recently reported a case of dengue fever in Tehran, which had been

imported from Malaysia (Kuala Lumpur). The mosquito-borne filariae *Dirofilaria* (dirofilariasis) (*D. immitis* and *D. repens*), *Setaria* (setariasis) (*S. labiatopapillosa*, *S. digitata*, and *S. equina*), and *Diptalonia evansi* (camel filariasis) (Spirurida: Onchocercidae) are found in Iran (Eslami, 1997; Azari-Hamidian et al., 2007; Oryan et al., 2008). *Anopheles maculipennis* Meigen and *Culex theileri* Theobald are known vectors of *Setaria labiatopapillosa* and *Dirofilaria immitis* respectively, in Ardebil Province, northwestern Iran (Azari-Hamidian et al., 2009). Malaria is the most important mosquito-borne disease, especially in the southeastern areas of Iran (Raeisi et al., 2004). Seven species are assumed to play a role as malaria vectors: *An. culicifacies* Giles sensu lato (s.l.), *An. dthali* Patton, *An. fluviatilis* s.l., *An. maculipennis* s.l., *An. sacharovi* Favre, *An. stephensi* Liston, and *An. superpictus* (Hanafi-Bojd et al., 2011). Zaim et al. (1993) also reported *An. pulcherrimus* Theobald as a potential vector in southeastern Iran. Eshghy (1977) observed *Plasmodium* oocysts in *An. multicolor* Cambouliu; however, sporozoites were not detected in this species, and it was not considered a vector in Iran. Djadid et al. (2009) recently reported *An. hyrcanus* (Pallas) as a

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possible vector of malaria using the nested polymerase chain reaction (PCR) technique in Guilan Province.

Malaria cases in Kurdistan Province, western Iran, increased from 1994 to 1996 (543, 633, and 236, respectively); however, they dramatically decreased after that. There were no cases of malaria in 2009, and just 2 and 3 cases in 2010 and 2011, respectively. All were imported from outside of the province (unpublished data from Department of Disease Control, Deputy of Health, Kurdistan University of Medical Sciences). Some areas of Kurdistan Province are classified in stratum 3 (with the imported cases of malaria, without potential indigenous transmission) and some areas in stratum 4 (without record of malaria during the past 3 years) in the National Malaria Strategy Plan for Malaria Control in Iran (Raeisi et al., 2004). West Nile virus has also been detected in equines in the province using serological assay (Ahmadnejad et al., 2011).

Until the present, 6 genera and 18 species of mosquitoes have been recorded in Kurdistan Province (Macan, 1950; Lotfi, 1973; Zaim, 1987; Vahabi, 2001; Moosa Kazemi et al., 2010). Seven species of *Anopheles* have been found in the province (Macan, 1950; Vahabi, 2001), including *An. algeriensis* Theobald, *An. claviger* (Meigen), *An. maculipennis* s.l., *An. marteri* Senevet and Prunelle, *An. sacharovi*, *An. sergentii* (Theobald), and *An. superpictus*. Of the *Maculipennis* group, *An. sacharovi* and *An. maculipennis* s.l. have been recorded in the province based on morphological characters. Zaim (1987) mentioned 6 species of *Culex* L. and 2 species of *Culiseta* Felt in the province: *Culex hortensis* Ficalbi, *Cx. mimeticus* Noe, *Cx. perexiguus* Theobald, *Cx. theileri*, *Cx. pipiens* L., *Cx. territans* Walker, *Culiseta longiareolata* (Macquart), and *Cs. subochrea* (Edwards). Moosa Kazemi et al. (2010) recently reported *Aedes vexans* (Meigen) and *Ochlerotatus caspius* (Pallas) s.l. in Kurdistan Province for the first time. Zaim and Cranston (1986) noted that a female specimen from Koolan of Marivan, Kurdistan Province, which had been previously identified by Lotfi (1973) as *Cx. impudicus* Ficalbi, was possibly a new species of *Coquillettidia* Dyar.

There have been only 2 recent studies on mosquitoes in Kurdistan Province, including the faunistic investigation of anophelines in the province by Vahabi et al. (2001) and that of culicines in Sanandaj County by Moosa Kazemi et al. (2010). There is very little information about the ecology of larvae, including their habitats, in the province and in a broader sense in western Iran. Ecological data, such as larval habitats, species composition, and active season, play an important role in integrated vector management. These data are used in source reduction through environmental manipulation and modification. In order to study the fauna and some aspects of the ecology of mosquito larvae, including habitats, species composition, association occasions, and percentages, an investigation was carried out in Kurdistan Province.

2. Materials and methods

2.1. Study area

Kurdistan Province is located in northwestern Iran between 34°44'N and 36°28'N and 45°33'E and 48°15'E and has mostly foothill and mountainous areas, with an area of approximately 29,137 km² (Figure). The province is bounded by West Azerbaijan and Zanjan provinces in the north, by Hamedan and Zanjan provinces in the east, by Kermanshah Province in the south, and by Iraq in the west. This province includes 10 counties: Baneh, Bijar, Dehgolan, Divandareh (Divandarreh, Diwandarreh), Kamyaran, Marivan, Qorveh (Qurveh), Sanandaj, Saqqez (Saqiz), and Sarvabad. The center of the province, Sanandaj City, is almost 1373 m above sea level. The average annual rainfall is about 460 mm. The average relative humidity changes are from 37% (1230 hours) to 69% (1830 hours). The averages of the maximum and minimum temperatures are 21.4 °C and 5.4 °C, respectively, and the average temperature is 13.4 °C (these averages are 44-year data from the Sanandaj Synoptic Station). The longtime average annual rainfall of the province is about 350 mm. The province includes cold arid and semiarid climates. Kurdistan Province has a population of almost 1,440,000 (43.6% in urban and

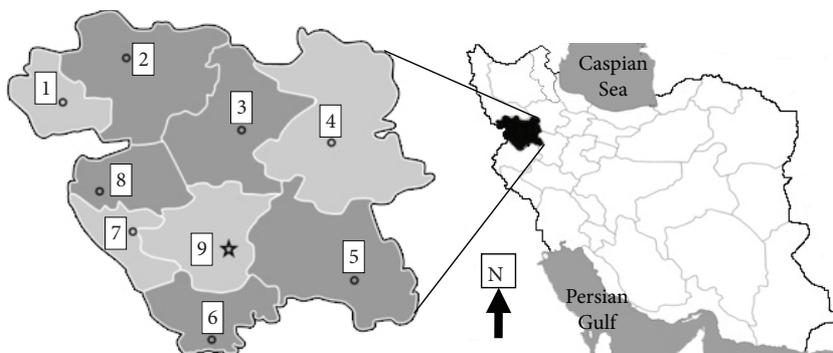


Figure. Map of Iran in which Kurdistan Province and its counties are highlighted: 1. Baneh, 2. Saqqez, 3. Divandareh, 4. Bijar, 5. Qorveh, 6. Kamyaran, 7. Sarvabad, 8. Marivan, 9. Sanandaj (* Sanandaj City).

57.4% in rural areas) according to the 2006 census. The main occupations in rural areas are agriculture and animal husbandry.

2.2. Specimen and data collection

To study mosquito fauna and ecology, larval sampling was carried out in Kurdistan Province by the dipping method (350-mL dipper) (WHO, 1975) during June–October 2005 and June–August 2006. The specimens were collected from 9 counties. The selected localities (cities and villages), occasions, and dates of collecting in each county in the different topographical and climatic areas of the province were: Baneh (Biandareh, 1 occasion, Jul 2006; Chooman, 4 occasions, Jul 2005, Jun–Jul 2006; Sardab, 2 occasions, Jul 2006), Bijar (Ghamchegha, 8 occasions, Aug–Oct 2005, Jun–Aug 2006; Khosroabad, 7 occasions, Aug–Oct 2005, Jun–Aug 2006), Dehgolan (Bag-e-jan, 3 occasions, Oct 2005, Jul 2006; Tahmasbgholi, 6 occasions, Sep–Oct 2005, Jun–Jul 2006), Divandareh (Gheshlaghsefid, 10 occasions, Jun–Aug and Oct 2005, Jun–Aug 2006; Hezarkanian, 11 occasions, Jul–Oct 2005, Jun–Aug 2006), Kamyaran (Islamabad, 5 occasions, Jun and Aug 2005, Jun–Jul 2006; Noshoor-sofla, 1 occasion, Aug 2006; Tangivar, 9 occasions, Jul–Sep 2005, Jun–Aug 2006), Qorveh (Farhadabad, 1 occasion, Aug 2005), Sanandaj (Barghoroo, 1 occasion, Jul 2006; Negel, 3 occasions, Jul–Aug 2006; Torivar, 5 occasions, Jul–Aug 2006; Vasi-olia, 1 occasion, Jun 2006; Vasi-sofla, 1 occasion, Jun 2006), Saqqez (Ghaghoolabad, 2 occasions, Jul 2006; Siahdar-sofla, 3 occasions, Aug 2005, Jun–Jul 2006; Yapeshkhan, 1 occasion, Jul 2006), and Sarvabad (Dezli, 9 occasions, Jul–Sep 2005, Jun–Aug 2006; Razab, 12 occasions, Jul–Sep 2005, Jun–Aug 2006). Physical and biological characteristics of larval habitats including habitat type, water condition including stability (stagnant or running) and turbidity (clear or turbid), water temperature, vegetation (with or without vegetation), and

sunlight exposure (full or partial sunlight or shaded) were recorded visually or using handheld field equipment. The continuous variable of the temperatures of larval habitats was analyzed by one-way ANOVA using SPSS 17 for Windows (SPSS Inc., Chicago, IL, USA). The larvae were temporarily preserved in lactophenol, and permanent microscope slides were prepared using de Faure's medium. The third- and fourth-instar larvae were identified using the keys of Azari-Hamidian and Harbach (2009). Mosquito name abbreviations follow Reinert (2009).

3. Results

In the present investigation, 2096 third- and fourth-instar larvae, including 1595 anopheline larvae (76.1%) and 501 culicine larvae (23.9%), were collected from 118 larval habitats in Kurdistan Province. Among 118 larval breeding sites, 61 (51.7%) contained only subfamily Anophelinae, 22 (18.6%) included only subfamily Culicinae, and 35 (29.7%) contained both subfamilies. In total, anopheline larvae were found in 96 oviposition sites (81.3%) and culicine larvae were found in 57 (48.3%).

Eleven species representing 4 genera were identified in the province, including *Anopheles claviger*, *An. maculipennis* s.l., *An. superpictus*, *Culex hortensis*, *Cx. mimeticus*, *Cx. perexiguus*, *Cx. pipiens*, *Cx. theileri*, *Culiseta longiareolata*, *Cs. subochrea*, and *Ochlerotatus caspius* s.l. (Table 1). *Anopheles superpictus* (57.7%), *An. maculipennis* s.l. (17.4%), and *Cx. theileri* (12.2%) were the most prevalent and widely distributed species. The association occasions of different mosquito larvae with other species are shown in Table 2. All larvae were collected from natural habitats (river edges or ground pools). No larvae were found in shaded habitats (Table 3). The association percentages of different mosquito larvae with other species are shown in Table 4. There was no significant difference

Table 1. Composition and localities of the larvae of mosquitoes collected in Kurdistan Province, western Iran, June–October 2005 and June–August 2006.

Species	County									n	%
	Baneh	Bijar	Dehgolan	Divandareh	Kamyaran	Qorveh	Sanandaj	Saqqez	Sarvabad		
<i>An. claviger</i>	2	13	-	-	-	-	5	-	1	21	1.0
<i>An. maculipennis</i> s.l.	29	45	15	217	26	-	2	13	17	364	17.4
<i>An. superpictus</i>	259	3	4	63	113	-	315	27	426	1210	57.7
<i>Cx. hortensis</i>	-	-	-	9	3	-	19	-	-	31	1.5
<i>Cx. mimeticus</i>	33	-	-	-	-	-	9	-	15	57	2.7
<i>Cx. perexiguus</i>	-	-	-	3	-	-	2	-	-	5	0.2
<i>Cx. pipiens</i>	-	-	12	4	-	-	-	-	-	16	0.8
<i>Cx. theileri</i>	6	36	19	82	9	9	9	22	64	256	12.2
<i>Cs. longiareolata</i>	-	112	-	-	-	-	17	-	-	129	6.1
<i>Cs. subochrea</i>	-	6	-	-	-	-	-	-	-	6	0.3
<i>Oc. caspius</i> s.l.	-	1	-	-	-	-	-	-	-	1	0.1
Total	329	216	50	378	151	9	378	62	523	2096	100

Table 2. The association occasions of different mosquito larvae with other species in Kurdistan Province, western Iran, June–October 2005 and June–August 2006.

Species	Total occasions	<i>An. claviger</i>	<i>An. maculipennis</i> s.l.	<i>An. superpictus</i>	<i>Cx. hortensis</i>	<i>Cx. mimeticus</i>	<i>Cx. perexiguus</i>	<i>Cx. pipiens</i>	<i>Cx. theileri</i>	<i>Cs. longiareolata</i>	<i>Cs. subochrea</i>	<i>Oc. caspius</i> s.l.
<i>An. claviger</i>	6	-	4	3	-	-	-	-	4	2	2	-
<i>An. maculipennis</i> s.l.	43	4	-	17	2	1	1	2	12	1	1	1
<i>An. superpictus</i>	64	3	17	-	10	5	2	4	14	2	-	-
<i>Cx. hortensis</i>	11	-	2	10	-	3	2	1	4	-	-	-
<i>Cx. mimeticus</i>	7	-	1	5	3	-	-	-	2	-	-	-
<i>Cx. perexiguus</i>	2	-	1	2	2	-	-	-	1	-	-	-
<i>Cx. pipiens</i>	6	-	2	4	1	-	-	-	5	-	-	-
<i>Cx. theileri</i>	32	4	12	14	4	2	1	5	-	1	2	1
<i>Cs. longiareolata</i>	9	2	1	2	-	-	-	-	1	-	-	-
<i>Cs. subochrea</i>	2	2	1	-	-	-	-	-	2	-	-	-
<i>Oc. caspius</i> s.l.	1	-	1	-	-	-	-	-	1	-	-	-

Table 3. The larval habitat characteristics and occurrence percentages of different mosquito larvae in Kurdistan Province, western Iran, June–October 2005 and June–August 2006.

Larval breeding site: characteristics and habitats	<i>An. claviger</i> (%)	<i>An. maculipennis</i> s.l. (%)	<i>An. superpictus</i> (%)	<i>Cx. hortensis</i> (%)	<i>Cx. mimeticus</i> (%)	<i>Cx. perexiguus</i> (%)	<i>Cx. pipiens</i> (%)	<i>Cx. theileri</i> (%)	<i>Cs. longiareolata</i> (%)	<i>Cs. subochrea</i> (%)	<i>Oc. caspius</i> s.l. (%)
A) Habitat type											
1. River edge	100	40.2	86.4	61.3	100	40	75.0	61.4	100	100	100
2. Ground pool	-	59.8	13.6	38.7	-	60	25.0	38.6	-	-	-
B) Water conditions											
1. Stagnant water	31.3	94.6	71.3	100	40.4	100	62.5	89.0	100	83.3	100
2. Slow-running water	68.7	5.4	28.7	-	59.6	-	37.5	11.0	-	16.7	-
3. Clear	43.8	98.8	95.5	96.8	100	100	100	96.2	100	83.3	100
4. Turbid	56.2	1.2	4.5	3.2	-	-	-	3.8	-	16.7	-
C) Vegetation											
1. With vegetation	100	38.6	48.0	3.2	78.9	-	68.8	44.1	100	100	100
2. Without vegetation	-	61.4	52.0	96.8	21.1	100	31.2	55.9	-	-	-
D) Sunlight exposure											
1. Full sunlight	6.3	5.8	57.3	71.0	73.7	40	75.0	23.3	-	-	-
2. Partial sunlight	93.7	94.2	42.7	29.0	26.3	60	25.0	76.7	100	100	100

Table 4. The association percentages of different mosquito larvae with other species in Kurdistan Province, western Iran, June–October 2005 and June–August 2006.

Species association	Abundance (%)		
An. claviger			
<i>Cx. theileri</i> , <i>Cs. subochrea</i>	9 (42.9)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. pipiens</i> , <i>Cx. theileri</i>	3 (9.7)
<i>An. maculipennis</i> s.l., <i>Cx. theileri</i> , <i>Cs. subochrea</i>	4 (19.0)	<i>An. superpictus</i> , <i>Cx. theileri</i>	2 (6.4)
<i>An. superpictus</i> , <i>Cs. longiareolata</i>	3 (14.3)	<i>An. superpictus</i> , <i>Cx. perexiguus</i>	1 (3.2)
<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. theileri</i> , <i>Cs. longiareolata</i>	2 (9.5)	Alone	1 (3.2)
<i>An. maculipennis</i> s.l., <i>Cx. theileri</i>	2 (9.5)	Total	31 (100)
<i>An. maculipennis</i> s.l., <i>An. superpictus</i>	1 (4.8)	Cx. mimeticus	
Total	21 (100)	<i>An. superpictus</i>	33 (57.9)
An. maculipennis s.l.		Alone	12 (21.1)
Alone	177 (48.6)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i>	6 (10.5)
<i>An. superpictus</i>	82 (22.5)	<i>Cx. theileri</i>	3 (5.3)
<i>An. superpictus</i> , <i>Cx. theileri</i>	28 (7.7)	<i>An. superpictus</i> , <i>Cx. hortensis</i>	2 (3.5)
<i>Cx. theileri</i>	25 (6.8)	<i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. theileri</i>	1 (1.7)
<i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. pipiens</i> , <i>Cx. theileri</i>	17 (4.7)	Total	57 (100)
<i>An. claviger</i> , <i>Cx. theileri</i>	13 (3.6)	Cx. perexiguus	
<i>An. superpictus</i> , <i>Cx. pipiens</i> , <i>Cx. theileri</i>	8 (2.2)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. theileri</i>	3 (60)
<i>An. claviger</i> , <i>An. superpictus</i>	4 (1.1)	<i>An. superpictus</i> , <i>Cx. hortensis</i>	2 (40)
<i>An. claviger</i> , <i>Cx. theileri</i> , <i>Cs. subochrea</i>	4 (1.1)	Total	5 (100)
<i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. perexiguus</i> , <i>Cx. theileri</i>	3 (0.8)	Cx. pipiens	
<i>An. claviger</i> , <i>An. superpictus</i> , <i>Cx. theileri</i> , <i>Cs. longiareolata</i>	1 (0.3)	<i>Cx. theileri</i>	7 (43.7)
<i>An. superpictus</i> , <i>Cx. mimeticus</i>	1 (0.3)	<i>An. superpictus</i>	4 (25.0)
<i>Cx. theileri</i> , <i>Oc. caspius</i> s.l.	1 (0.3)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. theileri</i>	2 (12.5)
Total	364 (100)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. theileri</i>	2 (12.5)
An. superpictus		<i>An. superpictus</i> , <i>Cx. theileri</i>	1 (6.3)
Alone	622 (51.4)	Total	16 (100)
<i>An. maculipennis</i> s.l.	98 (8.1)	Cx. theileri	
<i>Cx. hortensis</i> , <i>Cx. mimeticus</i> , <i>Cx. theileri</i>	74 (6.1)	Alone	91 (35.5)
<i>Cx. hortensis</i>	66 (5.4)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i>	39 (15.2)
<i>Cx. theileri</i>	66 (5.4)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. perexiguus</i>	18 (7.0)
<i>Cx. mimeticus</i>	63 (5.2)	<i>Cx. mimeticus</i>	17 (6.6)
<i>Cx. hortensis</i> , <i>Cx. perexiguus</i>	43 (3.6)	<i>An. superpictus</i>	16 (6.3)
<i>An. maculipennis</i> s.l., <i>Cx. mimeticus</i>	33 (2.7)	<i>An. maculipennis</i> s.l.	14 (5.5)
<i>An. claviger</i> , <i>Cs. longiareolata</i>	32 (2.6)	<i>An. maculipennis</i> s.l., <i>Oc. caspius</i> s.l.	11 (4.3)
<i>Cx. hortensis</i> , <i>Cx. theileri</i>	29 (2.4)	<i>Cx. pipiens</i>	11 (4.3)
<i>An. claviger</i> , <i>An. maculipennis</i> s.l., <i>Cx. theileri</i> , <i>Cs. longiareolata</i>	25 (2.1)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. pipiens</i>	9 (3.5)
<i>An. claviger</i> , <i>An. maculipennis</i> s.l.	18 (1.5)	<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. pipiens</i>	8 (3.1)
<i>Cx. hortensis</i> , <i>Cx. mimeticus</i>	18 (1.5)	<i>An. claviger</i> , <i>Cs. subochrea</i>	5 (1.9)
<i>An. maculipennis</i> s.l., <i>Cx. theileri</i>	10 (0.8)	<i>An. superpictus</i> , <i>Cx. pipiens</i>	4 (1.6)
<i>An. maculipennis</i> s.l., <i>Cx. pipiens</i> , <i>Cx. theileri</i>	5 (0.4)	<i>An. claviger</i> , <i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cs. longiareolata</i>	3 (1.2)
<i>An. maculipennis</i> s.l., <i>Cx. hortensis</i> , <i>Cx. perexiguus</i> , <i>Cx. theileri</i>	2 (0.2)	<i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. mimeticus</i>	3 (1.2)
<i>An. maculipennis</i> s.l., <i>Cx. hortensis</i> , <i>Cx. pipiens</i> , <i>Cx. theileri</i>	2 (0.2)	<i>An. superpictus</i> , <i>Cx. hortensis</i>	3 (1.2)
<i>Cx. pipiens</i>	2 (0.2)	<i>An. claviger</i> , <i>An. maculipennis</i> s.l., <i>Cs. subochrea</i>	2 (0.8)
Total	1210 (100)	<i>An. claviger</i> , <i>An. maculipennis</i> s.l.	2 (0.8)
Cx. hortensis		Total	256 (100)
<i>An. superpictus</i> , <i>Cx. mimeticus</i> , <i>Cx. theileri</i>	7 (22.6)	Cs. longiareolata	
<i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. perexiguus</i> , <i>Cx. theileri</i>	6 (19.4)	Alone	125 (96.9)
<i>An. superpictus</i> , <i>Cx. mimeticus</i>	6 (19.4)	<i>An. claviger</i> , <i>An. superpictus</i>	3 (2.3)
<i>An. superpictus</i>	5 (16.1)	<i>An. claviger</i> , <i>An. maculipennis</i> s.l., <i>An. superpictus</i> , <i>Cx. theileri</i>	1 (0.8)
		Total	129 (100)
		Cs. subochrea	
		<i>An. claviger</i> , <i>An. maculipennis</i> s.l., <i>Cx. theileri</i>	5 (83.3)
		<i>An. claviger</i> , <i>Cx. theileri</i>	1 (16.7)
		Total	6 (100)
		Oc. caspius s.l.	
		<i>An. maculipennis</i> s.l., <i>Cx. theileri</i>	1 (100)

among the mean water temperatures (17.7–26 °C) of the larval habitats of different species ($P = 0.098$).

3.1. *Anopheles claviger*

Anopheles claviger larvae were collected from 6 larval breeding sites (5.1% of the total and 6.2% of the anopheline larval habitats) in August 2005 and June–August 2006. The maximum and minimum water temperatures of larval habitats were 29 °C and 17 °C, respectively, and mean temperature was 23 °C (SD = 8.5) for 2 larval breeding sites. The collecting localities: Baneh (Sardab, 2 larvae), Bijar (Khosroabad, 13 larvae), Sanandaj (Torivar, 5 larvae), and Sarvabad (Razab, 1 larva).

3.2. *Anopheles maculipennis* s.l.

Anopheles maculipennis s.l. larvae were collected from 43 larval breeding sites (36.4% of the total and 44.8% of the anopheline larval habitats) in June–October 2005 and June–August 2006. The maximum and minimum water temperatures of larval habitats were 29 °C and 16 °C, respectively, and mean temperature was 22.4 °C (SD = 4.7) for 11 larval breeding sites. The collecting localities: Baneh (Chooman, 16 larvae; Sardab, 13 larvae), Bijar (Ghamcheghah, 26 larvae; Khosroabad, 19 larvae), Dehgolan (Tahmasbgholi, 15 larvae), Divandareh (Gheshlaghsefid, 92 larvae; Hezarkanian, 125 larvae), Kamyaran (Islamabad, 25 larvae; Tangivar, 1 larva), Sanandaj (Torivar, 2 larvae), Saqqez (Siahdar-sofla, 13 larvae), and Sarvabad (Razab, 17 larvae).

3.3. *Anopheles superpictus*

Anopheles superpictus larvae were collected from 64 larval breeding sites (54.2% of the total and 66.7% of the anopheline larval habitats) in July–October 2005 and June–August 2006. The maximum and minimum water temperatures of larval habitats were 32 °C and 19 °C, respectively, and mean temperature was 24.6 °C (SD = 3.6) for 22 larval breeding sites. The collecting localities: Baneh (Biandareh, 76 larvae; Chooman, 120 larvae; Sardab, 63 larvae), Bijar (Ghamchegha, 3 larvae), Dehgolan (Tahmasbgholi, 4 larvae), Divandareh (Gheshlaghsefid, 10 larvae; Hezarkanian, 53 larvae), Kamyaran (Islamabad, 8 larvae; Tangivar, 105 larvae), Sanandaj (Barghoroo, 13 larvae; Negel, 93 larvae; Torivar, 209 larvae), Saqqez (Ghagholabad, 1 larva; Siahdar-sofla, 20 larvae; Yapeshkhan, 6 larvae), and Sarvabad (Dezli, 189 larvae; Razab, 237 larvae).

3.4. *Culex hortensis*

Culex hortensis larvae were collected from 11 larval breeding sites (9.3% of the total and 19.3% of the culicine larval habitats) in July and August 2006. The maximum and minimum water temperatures of larval habitats were 29 °C and 24 °C, respectively, and mean temperature was 25.6 °C (SD = 1.9) for 5 larval breeding sites. The collecting localities: Divandareh (Gheshlaghsefid, 6 larvae;

Hezarkanian, 3 larvae), Kamyaran (Noshoor-sofla, 1 larva; Tangivar, 2 larvae), and Sanandaj (Barghoroo, 1 larva; Negel, 7 larvae; Torivar, 11 larvae).

3.5. *Culex mimeticus*

Culex mimeticus larvae were collected from 7 larval breeding sites (5.9% of the total and 12.3% of the culicine larval habitats) in July and August 2006. The maximum and minimum water temperatures of larval habitats were 28 °C and 25 °C, respectively, and mean temperature was 26 °C (SD = 1.7) for 3 larval breeding sites. The collecting localities: Baneh (Sardab, 33 larvae), Sanandaj (Negel, 1 larva; Torivar, 8 larvae), and Sarvabad (Dezli, 3 larvae; Razab, 12 larvae).

3.6. *Culex perexiguus*

Culex perexiguus larvae were collected from 2 larval breeding sites (1.7% of the total and 3.5% of the culicine larval habitats) in August 2006. The water temperature of 1 larval habitat was 25 °C. The collecting localities: Divandareh (Gheshlaghsefid, 3 larvae) and Sanandaj (Negel, 2 larvae).

3.7. *Culex pipiens*

Culex pipiens larvae were collected from 6 larval breeding sites (5.1% of the total and 10.5% of the culicine larval habitats) in October 2005 and July 2006. The collecting localities: Dehgolan (Bag-e-jan, 7 larvae; Tahmasbgholi, 5 larvae) and Divandareh (Gheshlaghsefid, 2 larvae; Hezarkanian, 2 larvae).

3.8. *Culex theileri*

Culex theileri larvae were collected from 32 larval breeding sites (27.1% of the total and 56.1% of the culicine larval habitats) in August–October 2005 and June–August 2006. The maximum and minimum water temperatures of larval habitats were 30 °C and 17 °C, respectively, and mean temperature was 23.6 °C (SD = 5.6) for 12 larval breeding sites. The collecting localities: Baneh (Chooman, 4 larvae; Sardab, 2 larvae), Bijar (Ghamchegha, 1 larva; Khosroabad, 35 larvae), Dehgolan (Bag-e-jan, 15 larvae; Tahmasbgholi, 4 larvae), Divandareh (Gheshlaghsefid, 56 larvae; Hezarkanian, 26 larvae), Kamyaran (Islamabad, 4 larvae; Tangivar, 5 larvae), Qorveh (Farhadabad, 9 larvae), Sanandaj (Torivar, 9 larvae), Saqqez (Ghagholabad, 10 larvae; Siahdar-sofla, 9 larvae; Yapeshkhan, 3 larvae), and Sarvabad (Dezli, 37 larvae; Razab, 27 larvae).

3.9. *Culiseta longiareolata*

Culiseta longiareolata larvae were collected from 9 larval breeding sites (7.6% of the total and 15.8% of the culicine larval habitats) in June–August 2006. The maximum and minimum water temperatures of larval habitats were 19 °C and 17 °C, respectively, and mean temperature was 17.7 °C (SD = 0.9) for 4 larval breeding sites. The collecting localities: Bijar (Ghamcheghah, 112 larvae) and Sanandaj (Torivar, 4 larvae; Vasi-olia, 12 larvae; Vasi-sofla, 1 larva).

3.10. *Culiseta subochrea*

Culiseta subochrea larvae were collected from 2 larval breeding sites (1.7% of the total and 3.5% of the culicine larval habitats) in June and July 2006. The water temperature of 1 larval habitat was 17 °C. The collecting locality: Bijar (Khosroabad, 6 larvae).

3.11. *Ochlerotatus caspius* s.l.

Ochlerotatus caspius s.l. larva was collected from 1 larval breeding site (0.8% of the total and 1.7% of the culicine larval habitats) in August 2006. The water temperature of the larval habitat was 18 °C. The collecting locality: Bijar (Khosroabad, 1 larva).

4. Discussion

Eleven species representing 4 genera were collected and identified in Kurdistan Province. *Anopheles superpictus*, *An. maculipennis* s.l., and *Cx. theileri* were the most abundant and widely distributed species (Tables 1 and 2).

Out of 7 species of *Anopheles* that had already been recorded in Kurdistan Province (Macan, 1950; Vahabi, 2001), 3 were collected in this investigation, including *An. claviger*, *An. maculipennis* s.l., and *An. superpictus*. Four species, *An. algeriensis*, *An. marteri*, *An. sacharovi*, and *An. sergentii*, were not found. In the previous study, Vahabi (2001) found only 1 adult *An. marteri* in the province, and *An. algeriensis* and *An. sergentii* were not common species. He also did not distinguish the *Maculipennis* group specimens in larval stage and identified the adults of *An. maculipennis* s.l. and *An. sacharovi* using morphological characters. In the present investigation, the larvae of the group were distinguished using the mean number of seta 2 branches of the fourth- and fifth-abdominal segments, which is 36.8 in *An. sacharovi* whereas it is 16.5 in *An. maculipennis* s.l. (Azari-Hamidian and Harbach, 2009). Based on the key, *An. sacharovi* was not found among the samples. There is no information about the group in the province using PCR techniques; however, *An. maculipennis* was found in the adjoining provinces of West Azerbaijan (Sedaghat et al., 2003) and East Azerbaijan (Djadid et al., 2007) based on molecular data. Furthermore, *An. maculipennis*, which has been identified using egg patterns, is a dominant species in eastern Turkey (Aldemir et al., 2009). Thus, the predominant species of the *Maculipennis* group in Kurdistan Province most probably is *An. maculipennis*. The genus *Coquillettia*, with its unique species *Cq. richiardii* (Ficalbi) in Iran, has been found in Ardebil, East Azerbaijan, Guilan, Mazandaran, and Kurdistan provinces (Zaim, 1987; Abai et al., 2007; Azari-Hamidian et al., 2009; Azari-Hamidian, 2011). Zaim and Cranston (1986) noted that the female specimen that had been previously identified by Lotfi (1973) as *Cx. impudicus* from Koolan of Marivan, Kurdistan Province, was possibly

a new species of *Coquillettia*. There is a *Coquillettia* female from Marivan in the Medical Arthropod Museum, School of Public Health, Tehran University of Medical Sciences without any other information on its label. Examination of this specimen revealed that it resembles specimens of *Cq. richiardii* from northern Iran (Azari-Hamidian and Harbach, 2009). Zaim and Cranston (1986) did not mention *Cx. impudicus* in their checklist of Iranian Culicinae. The occurrence of *Cx. impudicus* in Iran needs to be verified (Azari-Hamidian, 2007a; Azari-Hamidian and Harbach, 2009). *Coquillettia richiardii* was not found in this study. All records of *Cq. richiardii* in Iran are based on adult specimens; its larva, which pierces aquatic plants to obtain oxygen, has not been collected yet as the collectors did not search for larvae with such an adaptation. Five species of *Culex* were found in this study, out of 6 previously recorded species in the province (Zaim, 1987). *Culex territans* was not collected in this investigation. Moosa Kazemi et al. (2010) also did not find this species in Sanandaj County. Zaim (1987) introduced it as a relatively uncommon species. The species is also not abundant in Guilan Province (Azari-Hamidian, 2007b). Two species of *Culiseta* that were recorded in the province previously (Zaim, 1987), *Cs. longiareolata* and *Cs. subochrea*, were found in this investigation.

During the present study, *Anopheles superpictus* (57.7%), *An. maculipennis* s.l. (17.4%), and *Cx. theileri* (12.2%) were the most prevalent species in Kurdistan Province (Table 1). Vahabi (2001) also reported *Anopheles superpictus* (49.8%) and the *An. maculipennis* group (40.5%) as the most abundant anopheline species in the province. Mousakazemi et al. (2000) collected *An. maculipennis* s.l. (including 2.08% of the whole samples) and *An. superpictus* (0.16%) from rice fields in Lenjan and Mobarakeh areas (Isfahan Province, central Iran), while *Cx. theileri* (78.6%) was the most prevalent species. Ghavami and Ladonni (2006) reported *Cx. theileri* (68.2%) and *An. maculipennis* s.l. (16.3%) as being the most abundant species in both adult and larval stages in Zanjan Province, northwestern Iran. Abai et al. (2007) found *An. maculipennis* s.l. (14.4%) and *Cx. theileri* (24.0%) to be the most prevalent *Anopheles* and *Culex* species in larval stage in East Azerbaijan Province, northwestern Iran. Azari-Hamidian et al. (2009) collected *An. maculipennis* s.l. (19.0%) in Ardebil Province, northwestern Iran, more than any other anopheline species, while *Cx. theileri* (27.1%) was the most prevalent species in the province. Azari-Hamidian (2011) reported *An. maculipennis* s.l. (12.6% of the family) and *An. superpictus* (0.9%) as the most and the least abundant anopheline species, respectively, in Guilan Province in the Caspian Sea littoral, northern Iran; however, the most prevalent culicine species in the province was *Cx. pipiens*

(27.9%) (Azari-Hamidian, 2007b). In eastern Turkey, *An. maculipennis* and *Cx. theileri* are the most prevalent *Anopheles* and *Culex* species (Aldemir et al., 2009; Alkan and Aldemir, 2010). In agreement with previous investigations (Mousakazemi et al., 2000; Ghavami and Ladonni, 2006; Abai et al., 2007; Azari-Hamidian et al., 2009), the present study generally verifies that *Cx. theileri* is the most abundant *Culex* species in rural areas, at least in central and northern Iran, whereas *Cx. pipiens* is the most prevalent species in the cities and urban areas of the country and the lower-altitude Caspian Sea littoral (Azari-Hamidian, 2007b). There is little information about the composition percentages of the Pipiens subgroup in southern Iran. The taxonomy, ecology, and composition of *Cx. pipiens* and *Cx. quinquefasciatus* Say and the possibility of gene flow between them in southern Iran need to be studied extensively. On the other hand, while *An. maculipennis* group is the most prevalent anopheline species in some provinces in northern and northwestern Iran (Ghavami and Ladonni, 2006; Abai et al., 2007; Azari-Hamidian et al., 2009; Azari-Hamidian, 2011), *An. superpictus* is more abundant in Kurdistan Province, western Iran (Vahabi, 2001; and the present investigation), and it has been observed in some central provinces such as Isfahan (Doroudgar et al., 2000) and Qom (Saghafipour et al., 2012). Of course, any such conclusion needs to be verified with more quantitative surveillance of adults and larvae in different topographical regions.

In this investigation, all larvae were collected from natural habitats, including river edges and ground pools (Table 3). Among the 3 most prevalent species, *An. superpictus* was found along river edges (86.4%) with slow running water (28.7%) in full sunlight (57.3%) more often than the 2 other species, *An. maculipennis* s.l. and *Cx. theileri*. The edges of stony streams or rivers, especially with clear water and sunlight exposure, is noted as "the classic habitat" of *An. superpictus* (Macan, 1950), while *An. maculipennis* s.l. and *Cx. theileri* choose more varied natural and artificial habitats such as river edge pools, ground pools, and rice fields (Mousakazemi et al., 2000; Azari-Hamidian, 2007b, 2011). It is noteworthy that the types of larval habitats in a province such as Guilan (with about 1200 mm annual rainfall) are much more diverse than those of Kurdistan Province (about 350 mm annual rainfall) or other Iranian provinces where precipitation is low.

Another interesting finding in the present study is that the most prevalent collected species often occupied their habitats alone, as shown in Table 4: *An. maculipennis* s.l. (48.6%), *An. superpictus* (51.4%), *Cx. theileri* (35.5%), and *Cs. longiareolata* (96.9%). In view of the predation behavior of *Cs. longiareolata* larvae against other mosquito larvae (Shaalan, 2012), the high percentage of isolation of

the species can be explained, although this phenomenon was not observed in another study in Guilan Province, northern Iran (Azari-Hamidian, 2005). The difference may be a result of different altitude, climate, and other environmental parameters such as habitat type and water conditions (stability, turbidity, etc.), which influence larval abundance. *Culex pipiens*, the most prevalent species of the genus in Guilan Province, showed the highest percentage of having been collected in isolation (31.1%) (Azari-Hamidian, 2007b); however, the most abundant anopheline species, *An. maculipennis* s.l., *An. hyrcanus*, and *An. pseudopictus*, did not show this feature (Azari-Hamidian, 2011). It seems that less precipitation in a province such as Kurdistan makes mosquitoes compete more in choosing and occupying oviposition sites, in comparison with the higher precipitation of Guilan Province, where different natural and artificial larval habitats, especially rice fields, are available during the active season.

There are some potential vectors of human and domesticated animal pathogens, such as *An. maculipennis* s.l., *An. superpictus*, *Cx. pipiens*, *Cx. theileri*, and *Oc. caspius* s.l. (Azari-Hamidian et al., 2009; Hanafi-Bojd et al., 2011), among the mosquitoes of Kurdistan Province. The occurrence of some mosquito-borne infections such as malaria, West Nile, Sindbis (Sharifi et al., 2010; Ahmadnejad et al., 2011; Chinikar et al., 2011; Fereidouni et al., 2011), and an imported case of dengue fever in Iran (Chinikar et al., 2010), though there is no known vector of it in the country, shows the importance of mosquito and mosquito-borne disease surveillance.

There are some limitations in the present study that should be considered for forthcoming investigations: 1) The abundance and collecting occasions of some species, e.g. *Cx. perexiguus*, *Cs. subochrea*, and *Oc. caspius* s.l., were very low (Tables 1 and 2). More sampling should be done to assess their exact ecology and to analyze the continuous variables of habitats such as water temperature. 2) Because of some administrative limitations, sampling was not done or was incomplete in some localities such as Marivan and Qorveh counties. Such localities need to be investigated further in the future. 3) The species, including the *Maculipennis* group species, have been identified based on morphological characters; there is no information acquired by molecular data about the group in the province. 4) It is always possible to miss at least a part of very small larval habitats in the field during sampling. 5) The quantity and quality of larval habitats change due to different amounts of rainfall in different years, which may cause ecological data to vary through the years. 6) The first- and second-instar larvae, which are not reliably identifiable based on the available keys, might include other mosquito species. 7) It is not easy at all to compare the results of a study

designed as this one was with other investigations with different designs, those that are not as detailed as this one, or studies carried out in a very different climate, topography, or socioeconomic situation. 8) Larval habitat water pH, electrical conductivity, dissolved oxygen, other organic and inorganic compounds, and the presence of other aquatic associated animals (especially predators) are factors that should be considered in future studies.

All specimens are deposited in the Medical Arthropod Museum at the School of Public Health, Tehran University of Medical Sciences.

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