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Larval habitats, affinity and diversity indices of Culicinae (Diptera: Culicidae) in southern Iran

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Abstract
An investigation was carried out studying the ecology of the larvae of Culicinae (Diptera: Culicidae) in Bashagard County, Hormozgan Province, southern Iran. Larval habitat characteristics were recorded according to habitat situation and type, vegetation, sunlight situation, substrate type, turbidity and water depth during 2009–2011. Physicochemical parameters of larval habitat waters were analyzed for electrical conductivity (µS/cm), total alkalinity (mg/l), turbidity (NTU), total dissolved solids (mg/l), total hardness (mg/l), acidity (pH), water temperature (°C) and ions such as calcium, chloride, magnesium and sulphate. In total, 1479 third- and fourth-instar larvae including twelve species representing four genera were collected and identified: *Aedes vexans*, *Culex arbieeni*, *Cx. bitaeniorhynchus*, *Cx. mimeticus*, *Cx. perexiguus*, *Cx. quinquefasciatus*, *Cx. sinaiticus*, *Cx. theileri*, *Cx. tritaeniorhynchus*, *Culiseta longiareolata*, *Ochlerotatus caballus* and *Oc. caspius*. All species, except *Cx. bitaeniorhynchus*, were reported for the first time in Bashagard County. *Culiseta longiareolata* (37.5%), *Cx. sinaiticus* (23.7%), *Cx. quinquefasciatus* (22.9%) and *Cx. tritaeniorhynchus* (6.8%) were the most abundant species, respectively. *Culex sinaiticus*, *Cx. tritaeniorhynchus*, *Cs. longiareolata* and *Cx. perexiguus* showed the most distribution, respectively. The physicochemical features of habitat water, habitat characteristics, association occasions and percentages, affinity and diversity indices of the larvae are discussed.

Keywords: *Aedes*, *Culex*, *Culiseta*, *Ochlerotatus*, biodiversity

1. Introduction

Mosquito-borne diseases such as malaria, West Nile fever, and dirofilariasis are the most important vector-borne threats of human and domesticated animals in Iran [1–3]. Also, millions of people are threaten by different mosquito-borne diseases such as lymphatic filariasis, Japanese encephalitis, Rift Valley fever, and Yellow fever in other countries of the World Health Organization Eastern Mediterranean Region where includes Iran [4].

Hormozgan Province, southern Iran, is one of the most important areas of local transmission of malaria in Iran [2]. Mosquito-borne dirofilariasis is found in the province including a human case [1]. Azari-Hamidian *et al.* [5] have reported *Culex theileri* Theobald (Diptera: Culicidae) the vector of dirofilariasis in Ardebil Province, but there is no information about the vectors of the disease in other areas of the country. Ahmadnejad *et al.* [3] have reported West Nile virus in horses in Hormozgan Province using serological test, however there is no data about the vectors of the virus in the province.

The most recent checklist of Iranian mosquitoes includes seven genera and 64 species [6]. Since, Oshaghi *et al.* [7] introduced *Anopheles superpictus* Grassi as a species complex using Polymerase Chain Reaction (PCR) in which two species A and B were recognized later [8]. Also, Djadid *et al.* [9] introduced a new species of the Hyrcanus Group from southwestern Iran based on molecular data.

Six genera and forty species of mosquitoes are reported in Hormozgan Province including 13 species of *Anopheles* Meigen [10–19] and 27 culicine species [10, 12, 16, 20–23]. Among them, *An. claviger* (Meigen) and *An. subpictus* Grassi were recorded only by unpublished documents (the former Institute of Medical Parasiology and Tropical Medicine and former Institute of Public Health Research) and there is no recent published data on their occurrence in the province. McIntosh [24] has reported *Ochlerotatus chelli* (Edwards) in Djask County of Hormozgan Province, but there is no other record of the species in Iran and that is not mentioned in the checklist of Iranian mosquitoes [6].

Most of ecological data about mosquitoes in Hormozgan Province belong to anopheline species [10, 11, 14, 15, 17–19] especially malaria vectors such as *An. dthali* Patton [25, 26], *An. fluviatilis* James s.l. [27] and *An. stephensi* Liston [28–30]. Only Yaghoobi-Ershadi *et al.* [10] have studied the larval habitats of mosquitoes including the culicines in Minab County. Other published documents about Culicinae include only faunal records [12, 16, 20–23].

Regarding malaria problem, anopheline mosquitoes were recently studied in Bashagard County of Hormozgan Province and eight species of *Anopheles* were found [14, 15, 17, 19], but there is no published data about Culicinae in the county. In this regard only Yaghoobi-Ershadi *et al.* [10] recorded *Cx. bitaeniorhynchus* Giles in Bashagard.

Temperature, salinity and organic matter (for example ammonium ion), and pH (for some species) influence distribution of aquatic stages of mosquitoes [31, 32]. Some of other physicochemical characteristics are reported to show significant correlation with larval density of specific species. The examples are sodium and potassium [33], calcium [34] and dissolved oxygen [35]. Ecological data, such as physicochemical features of oviposition sites, larval habitat characteristics, species composition, and active season play an important role in integrated vector management (IVM). Those data help the managers to make the best decision in controlling the aquatic stages of vectors especially using source reduction through environmental manipulation and modification in addition to chemical and biological controls. The present study provides some ecological features of culicine larvae such as physicochemical factors of habitat water, habitat characteristics, associated species, affinity and diversity indices as well as fauna in Bashagard County of Hormozgan Province for the first time.

2. Materials and Methods

2.1. Study area

Bashagard County is located in latitude and longitude of 26º04'–26º58' N, 57º23'–52º02'E with a population of about 31,000 in the last national census in 2011. The county is a developing area in Hormozgan Province, southern Iran (Figure 1). Relative humidity and temperature range between 30–65% and 7–45 ºC, respectively, while average of annual rainfall and relative humidity are 265 mm and 40%, respectively. Population of this hilly county is scattered and mainly inhabited close to main and seasonal rivers. The soil texture is mainly sandy and is not able to preserve the precipitation as surface water.

2.2. Larval collection and identification

During 2009–2011, five villages and cities were selected as fixed sites for larval collection (Bolbolabad: 26º25'N 57º39'E, 680 m elevation; Chokhoon: 26º41'N 57º41'E, 450 m; Dargazan: 26º29'N 57º47'E, 1020 m; Molkan: 26º29'N 58º02'E, 920 m; Sardasht: 26º27'N 57º54'E, 710 m). Also random sampling was carried out in 11 other sites. The frequencies of collections in all of fixed and random sites were as follow: Ashkan: 1 (29 Oct 2009), Biskav: 1 (11 Apr 2010), Bolbolabad: 5 (3 Nov 2009, 18 Feb, 15 Apr, 25 May, 22 Jun 2010), Chokhoon: 7 (2 Nov 2009, 18 Feb, 28 March, 14 Apr, 27 May, 15 Sep, 12 Oct 2010), Daranar: 3 (20 Oct, 4 Nov 2009, 19 May 2010), Dargazan: 8 (3 Nov 2009, 19 Feb, 29 Mar, 13 Apr, 25 May, 22 Jun, 10 Aug, 15 Sep 2010), Goorichi: 3 (29 Oct, 2 Nov 2009, 19 March 2011), Islamabad: 1 (23 Oct 2009), Jakdan: 4 (14 Apr, 22 Jun, 15 Jul, 26 Jul 2010), Kahno: 1 (2 Nov 2009), Koohheidar: 1 (30 March 2010), Molkan: 10 (17 Feb, 12 Apr, 26 May, 23 June, 26 Jul, 15 Sep,11 Oct, 12 Nov, 14 Dec 2010, 28 Jan 2011), Nasary: 2 (6 Sep, 3 Nov 2009), Poshtgar: 2 (4 Nov 2009, 10 May 2010), Sardasht: 19 (21 Oct, 1 Nov 2009, 16 Feb, 19 Feb, 25 Mar, 11 Apr, 15 Apr, 12 May, 24 May, 22 Jun, 11 Jul, 26 Jul, 27 Aug, 14 Sep, 29 Sep, 7 Oct, 10 Oct, 14 Dec 2010, 24 Jan 2011), and Tisoor: 2 (6 Sep, 3 Nov 2009). All potential larval habitats of mosquitoes were sampled in the selected villages using a standard dipper (350 ml) [36]. Collected larvae were transferred to the laboratory of Malaria Research Center of Bashagard County and preserved in lactophenol. The third- and fourth-instar larvae were mounted by Berlese's medium and identified morphologically using taxonomic keys of Azari-Hamidian and Harbach [37]. Mosquito name abbreviations were cited based on Reinert [38].

2.3. Physicochemical analysis of water of larval habitats

For analysis of physicochemical characteristics, water samples were collected from different habitats in 1000 mL polyethylene bottles and transferred to the laboratory with cold boxes. The samples were analyzed for electrical conductivity (EC) (µS/cm), total alkalinity (mg/l), turbidity (NTU), total dissolved solids (TDS) (mg/l), total hardness (mg/l), acidity (pH), water temperature (°C) and ions such as calcium (Ca), chloride (Cl), magnesium (Mg) and sulphate (SO_4). Water temperature, pH, and turbidity were determined using thermometric, potentiometeric, and nephelometric methods, respectively. Alkalinity and total hardness were determined via titration while conductivity and TDS were measured using spectrophotometric technique. Spectrophotometer HachDR2800® (Hach Company, USA) was used for measurement of sulphate and chloride while calcium and magnesium were measured using Flame Atomic Absorption Spectrometry. Water quality chemical indicators were measured according to standard methods [39]. The temperature of water was measured for almost all larval habitats. Other physicochemical characteristics were measured for fourteen larval habitats of nine sites as follow: Ashkan: one time (29 Oct 2009), Daranar: 3 (20 Oct, 4 Nov 2009, 19 May 2010), Goorichi: 2 (29 Oct 2009, 19 March 2011), Islamabad: 1 (23 Oct 2009), Kahno: 1 (2 Nov 2009), Nasary: 2 (6 Sep, 3 Nov 2009), Poshtgar: 1 (4 Nov 2009), Sardasht: 1 (21 Oct 2009), and Tisoor: 2 (6 Sep, 3 Nov 2009).

2.4. Larval habitat characterization

Physical and biological characteristics of larval habitats were recorded visually based on habitat situation (temporary or permanent), habitat type (natural or man-made), aquatic vegetation (without vegetation or with vegetation including emergent, floating, submerged, or algae), sunlight situation (full, partial sunlight or shaded), substrate type (sand, mud, gravel, or cement), water turbidity (clear or turbid) and water salinity (fresh or brackish). The water depth of larval habitat was also recorded according to centimeters.

2.5. Indices of affinity and association between species

The indices of affinity and association between pairs of species were calculated using two formulae: Fager and McGowan's test of affinity and the index of association [40, 41].

Fager and McGowan's test of affinity is defined by $[J/(N_A N_B)^{1/2}] - 1/2(N_B)^{1/2}$ where J = number of joint occurrences, N_A = total number of occurrences of species A, N_B = total number of occurrences of species B, species are assigned to the letters so that $N_A \leq N_B$. The expressions of pairs of species which were equal to or higher than 0.5 were considered to show affinity [40]. The index of association is defined by I = 2[J/A+B–0.5] where J = the number of individuals of both species in samples where they occur together, A = the total number of individuals of species A in all samples, and B = the total number of individuals of species B in all samples. In this formula the numbers of species individuals are taken into consideration. The formula has a range of +1 to –1 [41].

2.6. Diversity of species

The Shannon-Weaver or Shannon-Wiener function (H') was used to calculate species diversity (richness) at five fixed study sites [41]. Shannon's index is defined by $H' = -\sum p_i Log p_i$ where p_i= the proportion of the number of individuals of species i in the total sample number. The maximum possible Shannon's Diversity can be calculated by $H'_{max} = Log_{10} k$ where k is the number of species collected in the sample. Evenness (J' or E or Pielou's index) can be calculated by $J' = H' / H'_{max}$.

2.7. Statistical analysis

The means of physicochemical parameters of water samples of the species were compared by Kruskal-Wallis test of nonparametric analysis using SPSS software (Version 16 for windows, SPSS Inc., Chicago, IL).

3. Results

During 70 rounds of collection, 103 larval habitats, in which 43 (41.7%) included culicine larvae, were sampled. In total, 1479 third- and fourth-instar larvae including twelve species representing four genera of Culicinae were collected and identified: *Aedes vexans* (Meigen), *Culex arbieeni* Salem, *Cx. bitaeniorhynchus*, *Cx. mimeticus* Noe, *Cx. perexiguus* Theobald, *Cx. quinquefasciatus* Say, *Cx. sinaiticus* Kirkpatrick, *Cx. theileri* Theobald, *Cx. tritaeniorhynchus* Giles, *Culiseta longiareolata* (Macquart), *Ochlerotatus caballus* (Theobald) and *Oc. caspius* (Pallas). All species, except *Cx. bitaeniorhynchus*, were reported for the first time in Bashagard County. *Culiseta longiareolata* (37.5%), *Cx. sinaiticus* (23.7%), *Cx. quinquefasciatus* (22.9%) and *Cx. tritaeniorhynchus* (6.8%) were the most prevalent species, respectively (Table 1). *Culex sinaiticus*, *Cx. tritaeniorhynchus, Cs. longiareolata* and *Cx. perexiguus* were most widely distributed species, respectively (Table 2). The number of larval habitats where each species was collected and its percentages in culicine larval habitats and total larval habitats were as follow: *Ae. vexans*: 2 (4.6%, 1.9%), *Cx. arbieeni*: 1 (2.3%, 1.0%), *Cx. bitaeniorhynchus*: 7 (16.3%, 6.8%), *Cx. mimeticus*: 2 (4.6%, 1.9%), *Cx. perexiguus*: 8 (18.6%, 7.8%), *Cx. quinquefasciatus*: 8 (18.6%, 7.8%), *Cx. sinaiticus*: 30 (69.8%, 29.1%), *Cx. theileri*: 1(2.3%, 1.0%), *Cx. tritaeniorhynchus*: 15 (34.9%, 14.6%), *Cs. longiareolata*:15 (34.9%, 14.6%), *Oc. caballus*:1 (2.3%, 1.0%) and *Oc. caspius*: 1 (2.3%, 1.0%).

3.1. Physicochemical analysis of water of larval habitats

The mean, standard deviation (SD) and range of physicochemical parameters such as acidity (pH), water temperature (°C), EC (µS/cm), TDS (mg/l), turbidity (NTU) and the water depth of larval habitat (cm) were shown for twelve species in table 3. Those of other parameters including total alkalinity, total hardness, and the amount of ions such as Ca, Cl, Mg and SO_4 were presented for seven species in table 4. The physicochemical parameters of water samples of the species did not show any significant differences ($P > 0.05$). Most of data for *Cx. bitaeniorhynchus*, *Cx. perexiguus*, *Cx. quinquefasciatus*, *Cx. sinaiticus* and *Cx. tritaeniorhynchus* were determined for the first time in Iran.

3.2. Larval habitat characterization

During the present study, the larvae of different species were collected from ten habitats including both natural (Ground pool, river bed pool, river edge, seepage, spring, stream edge) and man-made (Dyke leakage, palm field-palm irrigation channel, pool, seepage). The percentages of occurrence of four most prevalent species, *Cs. longiareolata*, *Cx. sinaiticus*, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus*, in natural habitats were 66.9%, 78.2%, 87.6% and 95.3, respectively. Larval habitat characteristics of the species were presented in table 5. Some larval habitat types were shown in figure 2.

3.3. Indices of affinity and association between species

The total numbers of individuals of each species used in the calculation of the index of association were available from table 1. The total occasions of twelve species and the numbers of co-occurrences of both species where occurred together were shown in table 6. The numbers of individuals of both species where they occurred together for twelve species were available from table 7. The available data in tables 6 and 7 were used to calculate Fager and McGowan's test of affinity and the index of association, respectively (Table 8). The pair of species *Cx. sinaiticus*/ *Cx. tritaeniorhynchus* showed significant affinity (0.521) according to Fager and McGowan's test. The pair of species *Ae. vexans*/ *Oc. caballus* showed significant association (0.784) using the index of association (Table 8).

3.4. Diversity of species

Shannon's diversity index (H'), the maximum possible Shannon's diversity (H'_{max}) and evenness (Pielou's index) (E or J') were calculated for five fixed localities (Bolbolabad, Chokhoon, Dargazan, Molkan and Sardasht) and presented in table 9. The index and evenness indices ranged from 0.301 and 0.500 in Bobolabad to 0.587 and 0.840 in Molkan, respectively. The maximum possible Shannon's diversity ranged from 0.602 in Bolbolabad to 0.845 in Dargazan (Table 9).

4. Discussion

During the present investigation, 1479 third- and fourth-instar larvae of Culicinae including twelve species and four genera were identified. Though, all of the species were reported from Hormozgan Province [10, 12, 16, 20–23], as far as the authors know only *Cx. bitaeniorhynchus* has been found in Bashagard County [10]. Thus, eleven species are reported for the first time in the county. Among them there are some important vectors of human and domesticated animal infections which are known to occur in Iran or neighboring countries such as *Ae. vexans* (Rift Valley fever), *Cx. bitaeniorhynchus* (Filariasis, Sindbis virus), *Cx. perexiguus* (Sindbis virus, West Nile fever), *Cx. quinquefasciatus* (Urban filariasis, Japanese encephalitis, West Nile fever), *Cx. theileri* (Dirofilariasis, Rift Valley fever, Sindbis virus, West Nile fever), *Cx. tritaeniorhynchus* (Japanese encephalitis, Rift Valley fever, Sindbis virus, West Nile fever), *Oc. caspius* (Rift Valley fever) [5, 42, 43].

4.1. Physicochemical analysis of water of larval habitats

Though, the physicochemical parameters of water samples of the species did not show significant differences ($P > 0.05$) (Tables 3 and 4), most of data for *Cx. bitaeniorhynchus*, *Cx. perexiguus*, *Cx. quinquefasciatus*, *Cx. sinaiticus*, and *Cx. tritaeniorhynchus* were become available for the first time in Iran. Because of some laboratory limitations, all physicochemical characteristics of water samples were not analyzed, thus for five species *Ae. vexans*, *Cx. mimeticus*, *Cx. theileri*, *Oc. caballus*, and *Oc. caspius*, only temperature and depth of larval habitat water were determined (Table 3). Thus, the numbers of frequency of some analyzed physicochemical features of *Cx. quinquefasciatus* and *Cs. longiareolata* were limited (Table 3 and 4). Also, the physicochemical parameter frequencies of *Cx. antennatus* were a few because of the low abundance of the species (Table 3 and 4). This phenomenon caused that the frequency of physicochemical features ranged from one to 29 for different species and the distribution of those features were not normal. That is why Kruskal-Wallis test of nonparametric analysis was used to assess data. There is little information about the physicochemical characteristics of water habitats of culicine larvae in Iran. In the most of previous investigations, the collecting data of larval habitats included only water temperature or pH (e.g. [44, 45]). Abai *et al.* [46] provided some of physicochemical features for 14 species including ten culicines in Qom Province in which five, *Cx. arbieeni*, *Cx. mimeticus*, *Cx. theileri*, *Cs. longiareolata*, and *Oc. caspius* were found in the present study (Table 3 and 4). Hanafi-Bojd *et al.* [14] and Soleimani-Ahmadi *et al.* [18, 19] have determined some physicochemical characteristics of water samples of anopheline larval habitats in studied localities in Bashagard and Rudan Counties of Hormozgan Province, but they did not report those characteristics for each species and no correlation was analyzed. The temperature of habitat water has a great influence on the development of mosquito larvae [31]. Though pH affects the distribution of some species, there is no evidence that pH is a limiting factor [32]. Salinity influences the distribution of mosquitoes. Most of mosquito larvae develop in fresh water. However, nearly 5% live in brackish or saline waters [32]. Organic matter, for example ammonium ion in sewage, can restrict larval breeding and few species survive in heavily polluted waters. Anopheline larvae mainly develop in clean water and rarely are found in polluted habitats which seem to be favorable for some culicines [31, 32]. Sasikumar *et al.* [33] showed that Na, K, and pH had significant correlation with the density of *Mansonia uniformis* (Theobald) and *Ma. annulifera* (Theobald) larvae, however Ca, Mg, and rainfall showed no significant relation in India. Piyaratne *et al.* [34] have found a positive correlation of *An. culicifacies* Giles s.l. and *An. varuna* Iyengar abundances only to temperature and calcium, respectively. Surendran and Ramasamy [35] observed a significant correlation of *An. culicifacies* species E abundance to dissolved oxygen. Some available data about correlation between distribution and abundance of larvae with specific ions are contradictory and there is not enough information about physicochemical parameters of larval habitats for many mosquito species. In addition to the biological differences of different species, the same species has a range of tolerance and sometimes show different correlation with physicochemical features [46]. More sampling and data are needed to analyze physicochemical parameters of Iranian Culicinae.

4.2. Larval habitat characterization

During the present study, the relative abundance percentages of four most prevalent species, *Cs. longiareolata*, *Cx. sinaiticus*, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus*, from natural habitats were 66.9, 78.2, 87.6 and 95.3, respectively (Figure 2). Azari-Hamidian [44] and Banafshi *et al.* [47] collected *Cs. longiareolata* 100% from natural habitats in Guilan and Kurdistan Provinces, respectively. Zaim [23] and Ladonni *et al.* [48] collected the species 78% and 70.7% from natural habitats. The difference may be explained by higher precipitation in Guilan and Kurdistan Provinces that provide more natural habitats during active season, while lower precipitation in other localities make mosquitoes choose any available habitats including man-made ones to lay eggs. The available comparable data for *Cx. sinaiticus* and *Cx. quinquefasciatus* only provided by Zaim [23] in which the percentage of those species in natural habitats were 52.6% and 59.1%, respectively. Though both species distribute in southern Iran, the difference between percentages of the present investigation (78.2% and 87.6%) and Zaim [23]'s results may be because of the sampling size. Azari-Hamidian [45] collected 80.5% of *Cx. tritaeniorhynchus* larvae from natural habitats in Guilan Province, however the percentage was 24 for Zaim [23]. Because the annual precipitation is low (265 mm) in Bashagard County and the soil texture is mainly sandy and is not able to preserve rain water, the main sources of water for larval habitats are rivers, streams and irrigation channels. That is why only one ground pool provided by rain water was found including the larvae of *Ae. vexans*, *Cs. longiareolata*, and *Oc. caballus* (Table 5).

4.3. Indices of affinity and association between species

During the present study, the pair of species *Cx. sinaiticus*/*Cx. tritaeniorhynchus* showed significant affinity (0.521) according to Fager and McGowan's test (Table 8). Two species occurred 13 times together, while they were collected 30 and 15 times in total, respectively (Table 6). Though the affinity was significant (≥ 0.5), it was not very much. However it may show the similar biological requirements of two species. Nagm *et al.* [49] used ≥ mean plus one standard deviation as a threshold value which was calculated 0.544 for their investigation. Although, the pair of species *Ae. vexans*/*Oc. caballus* (0.784) showed significant association using the index of association (Table 8), the number of larvae of *Ae. vexans* (11) and *Oc. caballus* (45) is a few (Table 7). More data is needed to improve such affinities. Hanafi-Bojd *et al.* [14] found affinity between eight pairs of anopheline species using Fager and McGowan's test in Bashagard County. Ladonni *et al.* [48] studied the affinity and association of 13 species including 8 culicine species using Fager's affinity index and the index of association. There is no more data about the affinity and association of mosquitoes in Iran. It is noteworthy that among the most abundant species of the present investigation, *Cs. longiareolata* was mostly collected alone (36.6%) more than any other species (Table 7). This phenomenon, which was observed by Banafshi *et al.* [47] in Kurdistan Province either (96.6% alone), may be explained by the predation behavior of *Cs. longiareolata* against other mosquito larvae. However, that was not observed in Guilan Province where 10.6% larvae were collected alone [44]. The difference may be because of other parameters which influence on larval population such as habitat and physicochemical characteristics.

4.4. Diversity of species

In the present study, Molkan Village showed the most diversity (H') and evenness (J') indices as much as 0.587 and 0.840, respectively and Sardasht City was the second one with H' = 0.540 and J' = 0.694 (Table 9). Hanafi-Bojd *et al.* [14] reported the most diversity of anophelines in Sardasht City (0.829) and Bolbolabad Village (0.718). They did not report J' index. When evenness index (J') was calculated based on available data provided by Hanafi-Bojd *et al.* [14], Sardasht City and Molkan Village showed the most evenness for anophelines as much as 0.918 and 0.796, respectively. There is little data about the diversity of mosquitoes in Iran. Nikookar *et al.* [50] calculated different alpha and beta diversity indices in Neka, northern Iran, with different climate and species composition. Available data for the diversity of mosquitoes is basic to more investigation using a standard sampling in different climates of the country. Nagm *et al.* [49] found that species diversity of *Anopheles* species is significantly higher in dry season than rainy season in Roraima, Brazil. It should also be investigated in southern Iran.

Table 1: The composition of culicine larvae in Bashagard County, Hormozgan Province, Iran, 2009–2011

Species	**n**	**%**
Aedes vexans	11	0.7
Culex arbieeni	4	0.3
Cx. bitaeniorhynchus	23	1.6
Cx. mimeticus	2	0.1
Cx. perexiguus	46	3.1
Cx. quinquefasciatus	339	22.9
Cx. sinaiticus	351	23.7
Cx. theileri	1	0.1
Cx. tritaeniorhynchus	101	6.8
Culiseta longiareolata	554	37.5
Ochlerotatus caballus	45	3.1
Oc. caspius	2	0.1
Total	1479	100

Table 2: The distribution of culicine larvae in Bashagard County, Hormozgan Province, Iran, 2009–2011

Species	**Locality**															
	Ashkan	**Biskav**	**Bolbolabad**	**Chokhoon**	**Daranar**	**Dargazan**	**Goorichi**	**Islamabad**	**Jakdan**	**Kahno**	**Koohheidar**	**Molkan**	**Nasary**	**Poshtgar**	**Sardasht**	**Tisoor**
Aedes vexans	-	-	-	✶	-	-	-	-	-	-	-	-	-	-	-	-
Culex arbieeni	-	-	✶	✶	-	-	-	-	-	✶	-	-	-	-	-	-
Cx. bitaeniorhynchus	-	-	-	-	✶	-	-	-	-	-	-	-	✶	✶	✶	✶
Cx. mimeticus	-	-	-	-	-	✶	-	-	-	-	-	-	-	-	-	-
Cx. perexiguus	-	-	✶	✶	✶	✶	✶	-	-	-	-	✶	✶	-	-	-
Cx. quinquefasciatus	-	-	-	-	-	✶	-	✶	-	-	✶	✶	-	-	✶	-
Cx. sinaiticus	✶	✶	✶	✶	✶	✶	✶	-	✶	✶	-	✶	✶	✶	✶	✶
Cx. theileri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✶	-
Cx. tritaeniorhynchus	-	-	-	-	✶	✶	✶	-	-	✶	-	✶	✶	✶	✶	✶
Culiseta longiareolata	-	-	✶	✶	-	✶	-	✶	✶	✶	✶	✶	-	-	✶	-
Ochlerotatus caballus	-	-	-	✶	-	-	-	-	-	-	-	-	-	-	-	-
Oc. caspius	-	-	-	-	-	✶	-	-	-	-	-	-	-	-	-	-

Table 3: The physicochemical parameters of habitat water of culicine larvae in Bashagard County, Hormozgan Province, Iran, 2009–2011

Species (Total number of occurrence)	Physicochemical parameters (Mean±SD, Minimum–Maximum, Number)					
	Acidity (pH)	Temperature (ºC)	Electrical conductivity (µS/cm)	Total dissolved solids (mg/l)	Turbidity (NTU)	Depth (cm)
Aedes vexans (2)	-	22.5±2.1, 21.0–24.0, 2	-	-	-	50.0±0.0, 50.0–50.0, 2
Culex arbieeni (1)	7.2	18	983.3	785.3	19.2	--
Cx. bitaeniorhynchus (7)	7.9±0.6, 7.2–8.6, 6	26.0±3.6, 20.0–30.0, 7	1954.2±479.5, 1310.5–2432.3, 6	1113.1±419.6, 567.8–1718.3, 6	8.5±0.6, 7.6–9.7, 6	10.0
Cx. mimeticus (2)	-	25.0±0.0, 25.0–25.0, 2	-	-	-	17.5±3.5, 15.0–20.0, 2
Cx. perexiguus (8)	8.1±0.4, 7.6–8.5, 4	26.1±3.6, 22.0–32.0, 8	2225.8±232.7, 1972.4–2432.3, 4	1262.9±357.7, 971.4–1718.3, 4	9.1±2.1, 7.3–12.3, 4	22.5±18.9, 10.0–50.0, 4
Cx. quinquefasciatus (8)	6.9	23.5±3.8, 19.0–29.0, 7	948.4	1221.5	9.4	16.4±8.9, 5.0–30.0, 7
Cx. sinaiticus (30)	7.9±0.5, 7.2–8.6, 12	25.5±4.2, 18.0–33.0, 29	1887.2±494.3, 983.3–2432.3, 12	1080.1±410.6, 498.3–1742.4, 12	9.7±3.1, 7.3–19.2,12	16.1±6.5, 10.0–30.0, 18
Cx. theileri (1)	-	22.0	-	-	-	30.0
Cx. tritaeniorhynchus (15)	7.9±0.5, 7.2–8.5, 8	24.8±4.6, 18.0–33.0, 15	1793.1±489.5, 983.3–2432.3, 8	1197.3±440.9, 567.8–1742.4, 8	10.5±3.5, 8.2–19.2, 8	14.2±5.3, 10.0–20.0, 7
Culiseta longiareolata (15)	7.0±0.2, 6.9–7.2, 2	22.3±3.2, 17.0–29.0, 14	965.8±24.6, 948.4–983.3, 2	1003.4±308.4, 785.3–1221.5, 2	14.3±6.9, 9.4–19.2, 2	24.6±17.9, 5.0–60.0, 13
Ochlerotatus caballus (1)	-	21.0	-	-	-	50.0
Oc. caspius (1)	-	28.0	-	-	-	10.0

Table 4: The physicochemical parameters of habitat water of culicine larvae in Bashagard County, Hormozgan Province, Iran, 2009–2011

Species (Total number of occurrence)	Physicochemical parameters (Mean±SD, Minimum–Maximum) mg/l					
	Total alkalinity	Total hardness	Calcium	Chloride	Magnesium	Sulphate
Culex arbieeni (1)	149.1	187.1	48.2	76.6	10.7	172.6
Cx. bitaeniorhynchus (6)	452.2±73.6, 329.4–524.2	263.0±116.3, 123.8–364.5	34.0±21.7, 19.3-45.8	148.6±65.6, 97.8–233.3	22.2±7.9, 14.2–36.4	354.5±108.4, 217.5–488.6
Cx. perexiguus (4)	468.3±63.4, 412.4–523.6	291.5±113.3, 123.8–361.3	22.0±5.4, 19.3–30.2	170.8±70.3, 105.8–232.8	27.4±15.0, 14.2–49.1	401.7±96.2, 314.8–486.7
Cx. quinquefasciatus (1)	143.3	353.8	60.5	65.6	35.8	164.4
Cx. sinaiticus (12)	432.0±107.0, 149.1–524.2	237.4±107.0, 123.8–364.5	31.3±16.6, 18.7–65.8	129.7±68.4, 10.5–233.3	23.5±11.1, 10.7–48.8	336.1±101.6, 172.6–488.6
Cx. tritaeniorhynchus (8)	395.6±114.3, 149.1–523.6	231.3±103.0, 123.8–361.3	35.4±18.9, 19.3–65.8	120.5±47.2, 76.6–232.8	23.0±13.0, 10.7–48.8	306.6±90.9, 172.6-483.5
Culiseta longiareolata (2)	146.2±4.1, 143.3–149.1	270.4±117.8, 187.1–353.8	54.3±8.6, 48.2–60.5	71.1±7.7, 65.6–76.6	23.2±17.7, 10.7–35.8	168.5±5.7, 164.4–172.6

Table 5: The larval habitat characteristics and occurrence percentages of culicine larvae in Bashagard County, Hormozgan Province, Iran, 2009–2011

Larval habitat characteristics	*Aedes vexans* (%)	*Culex arbieeni* (%)	*Cx. bitaeniorhynchus* (%)	*Cx. mimeticus* (%)	*Cx. perexiguus* (%)	*Cx. quinquefasciatus* (%)	*Cx. sinaiticus* (%)	*Cx. theileri* (%)	*Cx. tritaeniorhynchus* (%)	*Culiseta longiareolata* (%)	*Ochlerotatus caballus* (%)	*Oc. caspius* (%)
A) Habitat situation												
1. Temporary	45.5	-	13.0	50.0	3.8	61.0	54.4	100	21.2	63.9	100	100
2. Permanent	54.5	100	87.0	50.0	96.2	39.0	45.6	-	78.8	36.1	-	-
B) Vegetation situation												
1. Without vegetation	54.5	-	95.7	-	69.2	11.9	73.0	100	48.2	48.5	-	100
2. With vegetation												
2a) Emergent	45.5	-	-	-	-	-	14.7	-	-	13.5	100	-
2b) Floating	-	-	-	50.0	19.2	1.1	-	-	21.2	11.0	-	-
2c) Submerged	-	-	-	-	-	48.6	-	-	-	9.4	-	-
2d) Algae	-	100	4.3	50.0	11.6	38.4	12.3	-	30.6	17.6	-	-
C) Sunlight situation												

1. Full sunlight	45.5	-	100	50.0	53.8	63.3	73.7	100	89.4	55.6	100	-
2. Partial sunlight	54.5	100	-	50.0	46.2	26.0	19.3	-	8.2	44.4	-	-
3. Shaded	-	-	-	-	-	10.7	7.0	-	2.4	-	-	100
D) Substrate type												
1. Sand	54.5	-	82.6	50.0	80.8	87.6	54.4	-	63.5	39.9	-	-
2. Mud	45.5	100	17.4	50.0	3.8	12.4	33.7	100	20.0	25.1	100	100
3. Gravel	-	-	-	-	15.4	-	11.9	-	16.5	12.9	-	-
4. Cement	-	-	-	-	-	-	-	-	-	22.1	-	-
E) Turbidity												
1. Clear	54.5	100	100	100	100	50.8	92.3	-	97.6	42.4	-	-
2. Turbid	45.5	-	-	-	-	49.2	7.7	100	2.4	57.6	100	100
F) Water salinity												
1. Fresh	100	100	100	100	100	76.3	98.2	100	100	97.8	100	100
2. Brackish	-	-	-	-	-	23.7	1.8	-	-	2.2	-	-
G) Habitat type												
1. Natural habitat	100	100	100	50.0	96.2	87.6	78.2	-	95.3	66.9	100	-
1a. Ground pool	45.5	-	-	-	-	-	-	-	-	20.2	100	-
1b. River bed pool	-	-	21.7	-	36.0	0.6	70.9	-	49.4	29.6	-	-
1c. River edge	-	100	65.2	-	8.0	-	7.2	-	12.3	0.4	-	-
1d. Seepage	-	-	-	-	-	0.6	4.0	-	-	-	-	-
1e. Spring	54.5	-	-	-	40.0	43.6	3.1	-	17.3	43.2	-	-
1f. Stream edge	-	-	13.1	100	16.0	55.2	14.8	-	21.0	6.6	-	-
2. Man-made habitat	-	-	-	50.0	3.8	12.4	21.8	100	4.7	33.1	-	100
2a. Dyke leakage	-	-	-	-	-	-	67.8	-	-	-	-	-
2b. Palm field	-	-	-	100	100	100	30.6	-	100	33.3	-	100
2c. Pool	-	-	-	-	-	-	-	-	-	66.7	-	-
2d. Seepage	-	-	-	-	-	-	1.6	100	-	-	-	-

Table 6: The association occasions of culicine larvae in Bashagard County, Hormozgan Province, Iran, 2009–2011

Species	**Total occasions**	*Aedes vexans*	*Culex arbieeni*	*Cx. bitaeniorhynchus*	*Cx. mimeticus*	*Cx. perexiguus*	*Cx. quinquefasciatus*	*Cx. sinaiticus*	*Cx. theileri*	*Cx. tritaeniorhynchus*	*Culiseta longiareolata*	*Ochlerotatus caballus*	*Oc. caspius*
Aedes vexans	2	-[*]	-	-	-	1	-	-	-	-	2	1	-
Culex arbieeni	1	-	-	-	-	-	-	1	-	1	1	-	-
Cx. bitaeniorhynchus	7	-	-	-	-	2	-	7	-	5	-	-	-
Cx. mimeticus	2	-	-	-	-	1	1	1	-	1	1	-	-
Cx. perexiguus	8	1	-	2	1	1	2	4	-	5	4	-	-
Cx. quinquefasciatus	8	-	-	-	1	2	1	3	1	2	5	-	-
Cx. sinaiticus	30	-	1	7	1	4	3	8	1	13	4	-	1
Cx. theileri	1	-	-	-	-	-	1	1	-	-	-	-	-
Cx. tritaeniorhynchus	15	-	1	5	1	5	2	13	-	-	4	-	-
Culiseta longiareolata	15	2	1	-	1	4	5	4	-	4	4	1	-
Ochlerotatus caballus	1	1	-	-	-	-	-	-	-	-	1	-	-
Oc. caspius	1	-	-	-	-	-	-	1	-	-	-	-	-

[*] Dash (-) means the pair of species did not occur together

Table 7: The association percentages of culicine larvae in Bashagard County, Hormozgan Province, Iran, 2009–2011

Species association	**n**	**Abundance (%)**
Ae. vexans		
Cx. perexiguus, Cs. longiareolata	6	54.5
Cs. longiareolata, Oc. caballus	5	45.5
Total	11	100
Cx. arbieeni		
Cx. sinaiticus, Cx. tritaeniorhynchus, Cs. longiareolata	1	100
Cx. bitaeniorhynchus		
Cx. sinaiticus, Cx. tritaeniorhynchus	19	82.6
Cx. perexiguus, Cx. sinaiticus, Cx. tritaeniorhuynchus	2	8.7

Cx. sinaiticus	2	8.7
Total	23	100
Cx. mimeticus		
Cx. perexiguus, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cs. longiareolata	1	50.0
Cx. sinaiticus	1	50.0
Total	2	100
Cx. perexiguus		
Ae. vexans, Cs. longiareolata	9	34.6
Cx. sinaiticus	5	19.3
Cx. bitaeniorhynchus, Cx. sinaiticus, Cx. tritaeniorhynchus	4	15.4
Cx. tritaeniorhynchus, Cs. longiareolata	4	15.4
Alone	2	7.7
Cx. quinquefasciatus,Cx. sinaiticus, Cx. tritaeniorhynchus, Cs. longiareolata	1	3.8
Cx. mimeticus, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cs. longiareolata	1	3.8
Total	26	100
Cx. quinquefasciatus		
Cx. sinaiticus, Cx. theileri	102	36.6
Cs. longiareolata	87	31.2
Cx. perexiguus, Cx. sinaiticus, Cx. tritaeniorhuynchus, Cs. longiareolata	68	24.4
Alone	19	6.8
Cx. mimeticus, Cx. perexiguus, Cx. tritaeniorhuynchus, Cs. longiareolata	2	0.7
Cx. sinaiticus	1	0.3
Total	279	100
Cx. sinaiticus		
Alone	79	27.5
Cs. longiareolata	39	13.6
Cx. bitaeniorhynchus	36	12.5
Cx. bitaeniorhynchus, Cx. perexiguus, Cx. tritaeniorhynchus	32	11.1
Cx. tritaeniorhynchus	28	9.8
Cx. bitaeniorhynchus, Cx. tritaeniorhynchus	21	7.3
Cx. perexiguus	20	7.0
Oc. caspius	19	6.6
Cx. perexiguus,Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cs. longiareolata	7	2.4
Cx. mimeticus	3	1.0
Cx. arbieeni, Cx. tritaeniorhynchus, Cs. longiareolata	1	0.4
Cx. quinquefasciatus, Cx. theileri	1	0.4
Cx. quinquefasciatus	1	0.4
Total	287	100
Cx. theileri		
Cx. quinquefasciatus, Cx. sinaiticus	1	100
Cx. tritaeniorhynchus		
Cx. bitaeniorhynchus, Cx. sinaiticus	18	21.1
Cx. bitaeniorhynchus,Cx. perexiguus, Cx. sinaiticus	17	20.0
Cx. sinaiticus	17	20.0
Cx. perexiguus, Cx. quinquefasciatus,Cx. sinaiticus, Cs. longiareolata	14	16.5
Cx. perexiguus, Cs. longiareolata	14	16.5
Cx. mimeticus, Cx. perexiguus, Cx. quinquefasciatus, Cs. longiareolata	4	4.7
Cx. arbieeni, Cx. sinaiticus, Cs. longiareolata	1	1.2
Total	85	100
Cs. longiareolata		
Alone	155	36.6
Cx. perexiguus, Cx. quinquefasciatus,Cx. sinaiticus, Cx. tritaeniorhynchus	63	14.9
Cx. perexiguus,Cx. tritaeniorhynchus	60	14.2
Ae. vexans, Oc. caballus	49	11.6
Ae. vexans, Cx. perexiguus	42	9.9
Cx. mimeticus, Cx. perexiguus, Cx. quinquefasciatus, Cx. tritaeniorhynchus	40	9.5
Cx. quinquefasciatus	11	2.6
Cx. sinaiticus	2	0.5
Cx. arbieeni, Cx. sinaiticus, Cx. tritaeniorhynchus	1	0.2
Total	423	100
Oc. caballus		
Ae. vexans, Cs. longiareolata	45	100
Oc. caspius		
Cx. sinaiticus	2	100

Table 8: Fager and McGowan's test of affinity and the index of association of culicine larvae in Bashagard County, Hormozgan Province, Iran, 2009–2011. Lower part shows the index of association. Upper part shows Fager and McGowan's test of affinity.

Fager and McGowan's testof Affinity Index of Association	*Aedes vexans*	*Culex arbieeni*	*Cx. bitaeniorhynchus*	*Cx. mimeticus*	*Cx. perexiguus*	*Cx. quinquefasciatus*	*Cx. sinaiticus*	*Cx. theileri*	*Cx. tritaeniorhynchus*	*Culiseta. longiareolata*	*Ohlerotatus. caballus*	*Oc. caspius*
Aedes vexans		-[a]	-	-	0.073	-	-	-	-	0.236	0.355	-
Culex arbieeni	-		-	-	-	-	0.091	-	0.129	0.129	-	-
Cx. bitaeniorhynchus	-	-		-	0.091	-	0.392	-	0.358	-	-	-
Cx. mimeticus	-	-	-		0.074	0.074	0.038	-	0.053	0.053	-	-
Cx. perexiguus	–0.190	-	–0.756	–0.858		0.074	0.167	-	0.327	0.236	-	-
Cx. quinquefasciatus	-	-	-	–0.980	–0.528		0.102	0.177	0.053	0.327	-	-
Cx. sinaiticus	-	–0.988	–0.278	–0.974	–0.560	–0.364		0.091	0.521[b]	0.097	-	0.091
Cx. theileri	-	-	-	-	-	–0.266	–0.988		-	-	-	-
Cx. tritaeniorhynchus	-	–0.954	0.036	–0.886	0.062	–0.518	–0.162	-		0.137	-	-
Culiseta longiareolata	–0.530	–0.992	-	–0.808	–0.022	–0.476	–0.682	-	–0.620		0.129	-
Ochlerotatus caballus	0.784[b]	-	-	-	-	-	-	-	-	–0.600		-
Oc. caspius	-	-	-	-	-	-	–0.856	-	-	-	-	

[a] Dash (-) means the pair of species did not occur together.

[b] Bold numbers show significant affinity and association.

Table 9: Shannon diversity index of culicine larvae in five fixed localities in Bashagard County, Hormozgan Province, Iran, 2009–2011

Village	**Species**	**Number**	p_i	**Log** p_i	p_i **Log** p_i	**H'**	**H'**$_{max}$	**J'**
Bolbolabad	*Cx. arbieeni*	2	0.010	–2	–0.020	0.301	0.602	0.500
	Cx. perexiguus	1	0.005	–2.301	–0.011			
	Cx. sinaiticus	60	0.304	–0.517	–0.157			
	Cs. longiareolata	134	0.680	–0.167	–0.113			
Chokhoon	*Ae. vexans*	11	0.062	–1.207	–0.074	0.531	0.778	0.682
	Cx. arbieeni	1	0.005	–2.301	–0.011			
	Cx. perexiguus	9	0.050	–1.301	–0.065			
	Cx. sinaiticus	14	0.079	–1.102	–0.087			
	Cs. longiareolata	97	0.548	–0.261	–0.143			
	Oc. caballus	45	0.254	–0.595	–0.151			
Dargazan	*Cx. mimeticus*	2	0.005	–2.301	–0.011	0.518	0.845	0.613
	Cx. perexiguus	4	0.011	–1.958	–0.021			
	Cx. quinquefasciatus	135	0.372	–0.429	–0.159			
	Cx. sinaiticus	35	0.096	–1.017	–0.097			
	Cx. tritaeniorhynchus	18	0.049	–1.309	–0.064			
	Cs. longiareolata	166	0.458	–0.339	–0.155			
	Oc. caspius	2	0.005	–2.301	–0.011			
Molkan	*Cx. perexiguus*	21	0.144	–0.841	–0.121	0.587	0.698	0.840
	Cx. quinquefasciatus	2	0.013	–1.886	–0.024			
	Cx. sinaiticus	31	0.213	–0.671	–0.142			
	Cx. tritaeniorhynchus	31	0.213	–0.671	–0.142			
	Cs. longiareolata	60	0.413	–0.384	–0.158			
Sardasht	*Cx. bitaeniorhynchus*	15	0.048	–1.318	–0.063	0.540	0.778	0.694
	Cx. quinquefasciatus	159	0.512	–0.290	–0.148			
	Cx. sinaiticus	31	0.100	–1.000	–0.100			
	Cx. theileri	1	0.003	–2.522	–0.007			
	Cx. tritaeniorhynchus	17	0.054	–1.267	–0.068			
	Cs. longiareolata	87	0.280	–0.552	–0.154			

Fig 1: Map of governmental provinces of Iran, Bashagard County is highlighted in Hormozgan Province

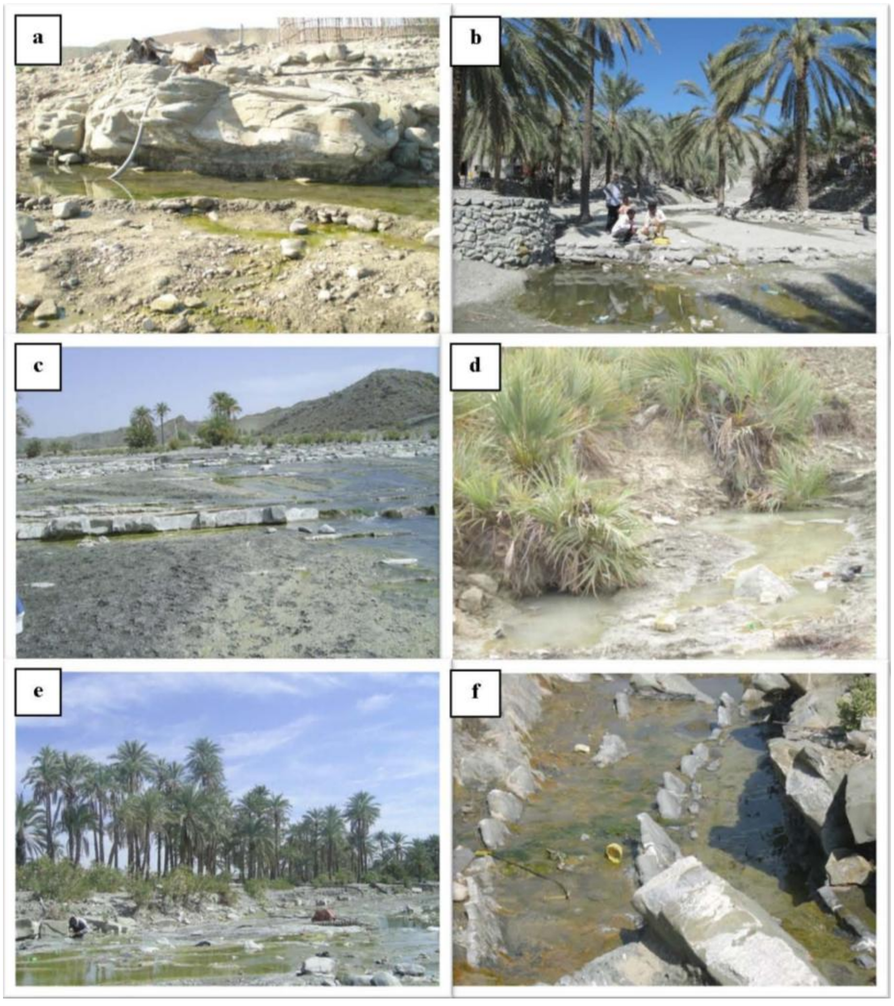

Fig 2: Six localities of larval collection in Bashagard County, Hormozgan Province, Iran, 2009–2011. (a) Daranar, River bed. (b) Dargazan, Palm field. (c) Molkan, River bed. (d) Nasary, River bed. (e) Sardasht, River bed. (f) Tisoor, River bed (Original photographs)

Conclusion
There is little information about the ecology of culicine larvae in Iran and southwestern Asia, though culicine-borne infections, including dirofilariasis, West Nile fever etc., are reported in the region. Available data is mostly in relation to anopheline malaria vectors. More studies are needed to do any further analysis and assessment. Such data help the managers to make the best decision in decreasing the aquatic stages of mosquitoes using environmental manipulation and modification.

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