

Species Diversity and Distribution Pattern of Anopheline Mosquitoes (Diptera: Culicidae) in Qaleh Ganj County, Southeast of Iran

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Abstract

Background: Malaria is the most important mosquito-borne disease in Iran in recent decades. This disease is endemic in the south to the southeastern Iran. Knowledge about larval habitats, species diversity, and distribution pattern of malaria vectors will help authorities in the appropriate management of this disease. Qaleh Ganj county is one of the main endemic areas for malaria in the south of Iran. Little information is available about the fauna of mosquitoes and their characteristics in this area.

Methods: This study aims to assess the fauna, larval habitats, species diversity, and distribution pattern of Anophelinae mosquitoes using the Geographic Information System (GIS) in Qaleh Ganj County, the southeast of Iran. The potential aquatic habitats for *Anopheles* larvae were selected based on the variety of topography and history of the malaria epidemic. The mosquito larvae were collected using dipping method from April to December 2018. Finally, the diversity, richness, and evenness indices were calculated.

Results: A total of 1042 larvae were found. The dominant species was *Anopheles culicifacies* s.l. Giles, 1901. The Shannon diversity index ranged from 0.76–1.54 between different collection sites, while the other indices showed the highest and lowest diversity, evenness, and richness of species in each area.

Conclusion: Due to the fact that some of the species in this region are species complexes, molecular studies are needed to find better information about the genetic diversity of these species which can be helpful for the malaria elimination program in Iran.

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Keywords: *Anopheles*, Species composition, Malaria, Larval habitat, Iran

Introduction

Malaria is one of the most important vector-borne diseases, especially in the tropical and subtropical regions of the world.¹ It has been reported from 87 countries with 229 million estimated cases and 409000 estimated deaths in 2019. The majority of malaria cases and deaths occurred in the African region of the World Health Organization (WHO).² Iran is situated in the Middle East region, which is one of the most important

malaria-prone areas in the world.³ This disease is one of the headmost public health concerns in Iran. More than 90 percent of malaria cases have been reported from the southern and south-eastern areas of Iran, including Sistan & Baluchistan, Hormozgan, and Kerman provinces.^{3,4}

Around 40 species of *Anopheles* act as the main malaria vectors all over the world.⁵ According to documented literature, seven species of *Anopheles* have been identified as malaria vectors in Iran including *An. stephensi*, *An. maculipennis*, *An. fluviatilis* s.l., *An.*

superpictus s.l., *An. dthali*, *An. culicifacies* s.l., and *An. sacharovi*.⁶ Some of the malaria vectors such as *An. maculipennis*, *An. culicifacies*, and *An. fluviatilis* have been recognized as the species complex.

Each species has individual needs and habitats.⁷ Larval control by larviciding and managing the environment is the main method for malaria vector control around the world.⁸ Identifying the mosquito larval habitats is critical in all control programs.⁸ Southern parts of Kerman Province including Jiroft, Kahnooj, Anbar Abad, Faryab, Roodbar, Manoojan, and Qaleh Ganj counties have been identified as endemic foci of malaria. Therefore, understanding the characteristics of the mosquitoes' larval habitats, their species diversity, and distribution pattern will be necessary for vector control programs as the main strategy in the elimination phase in this malaria-prone area of Iran.⁹ Qaleh Ganj County is an endemic malaria region, and a few researches have been conducted on the biology and ecology of malaria vectors in this area in the past.^{10, 11} Also, some studies with a short interval from this study have been conducted recently in this area.^{12, 13}

This study aimed to assess the fauna, larval habitats, species diversity, and distribution pattern of Anophelinae mosquitoes using the Geographic Information System (GIS) in Qaleh Ganj County, the southeast of Iran.

Methods

Study Area

This study was carried out in Qaleh Ganj County, in Kerman province from April to December 2018.

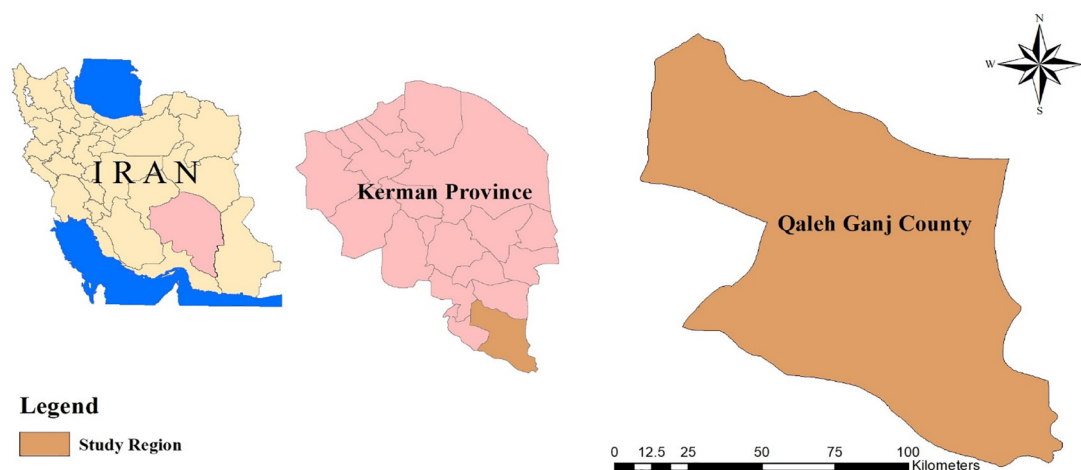


Figure 1: Location of the study area in Kerman Province (Designed by: Alireza Sanei-Dehkordi).

Table 1: Geographical characterization of the study area in Qaleh Ganj County

Location	Latitude	Longitude	Altitude (m)	Topography	Kind of district
Shahkahan	26°58' 11" N	57° 57' 13" E	1026	Mountainous	Elevated village with palm groves
Rameshk	26°50'14" N	58°48'37" E	666	Foothill	Mound village with palm groves
Deh bala	26°48'05" N	58° 49' 52" E	713	Sub mountain	Semi elevated village with palm groves
Galoo	27° 47'28" N	57° 57'03" E	442	Plain	Flat village with farms

Qaleh Ganj County is located in latitude 27° 32' N and longitude 57° 50' E with a population of more than 75000 in 2016. It is an underdeveloped area in Kerman province, southeastern of Iran (Figure 1). The relative humidity is between 1 and 35 percent and the temperature ranges from 2 to 52 °C, while the average annual rainfall is 162.8 mm.

The study locations have been pointed by the Global Positioning System (GPS) device (Garmin76CS). All locations are shown in Figure 1, using Arc map software, version 10.8 (Redlands, CA).

Sampling and Data Collection

The mosquito larvae were collected by dipping method from April to December 2018. Samples were collected from different geographical localities including Shahkahan, Deh Bala, Rameshk, and Galoo districts (Table 1), and then transferred to the laboratory of Biology and Vector control Department of Kerman University of Medical Sciences. The specimens were identified to the species level using standard taxonomic keys.¹⁴

Physicochemical Characteristics of Larval Habitats

Characteristics of each habitat including water depth, turbidity, and/or emergent vegetation, light/shadow, temperature, humidity, and pH were recorded in all sampling times. The geographic coordinate of each habitat was recorded by the GPS device.

Indices of Biodiversity and Species Co-occurrence

The Margalef's diversity index¹⁵ was calculated

for this research. It determines the species richness of each habitat. This index is calculated by the following equation:

$$R = \frac{S - 1}{\ln N}$$

Where S is the number of species, and N is the number of individuals. Simpson's diversity index¹⁶ is calculated using the following equation:

$$D = \sum_{i=1}^S \frac{n_i (n_i - 1)}{N(N - 1)}$$

where N is the total number of mosquitoes of all species and n is the total number of flies of a particular species. Shannon diversity index¹⁷ was calculated using the following equation:

$$H' = \sum_{i=1}^S (p_i) [\ln(p_i)]$$

where H' is the Shannon diversity index, Pi is the fraction of the entire population made up of species i, S is the number of species encountered, and Σ is the sum from species 1 to species. Pielou's evenness index¹⁸ was calculated by the following equation:

$$E = \frac{H'}{\ln(s)}$$

where E is the Pielou's evenness index, H is the calculated Shanon-Wiener index, and s is the number of species in the community.

Results

Species Composition of Anopheles Larvae

In this study, totally 1042 *Anopheles* larvae were collected and identified. They comprised of seven species including *An. culicifacies* s.l. (29.75%), *An. dthali* (28.7%), *An. moghulensis* (16.31%), *An. stephensi* (14.78%), *An. superpictus* s.l. (8.25%), *An. fluviatilis* s.l. (1.15%), and *An. turkhudi* (1.06%) (Figure 2). The results of this study showed that the *An. culicifacies* s.l. Giles, 1901 was the most abundant species among the collected ones in Qaleh Ganj. Spatial distribution of the collected species of *Anopheles* in our study area are illustrated in Figure 3.

Physicochemical Characteristics of Larval Habitats

In this study, 20 larval habitats in four districts including Shahkahan, Deh Bala, Rameshk, and Galoo

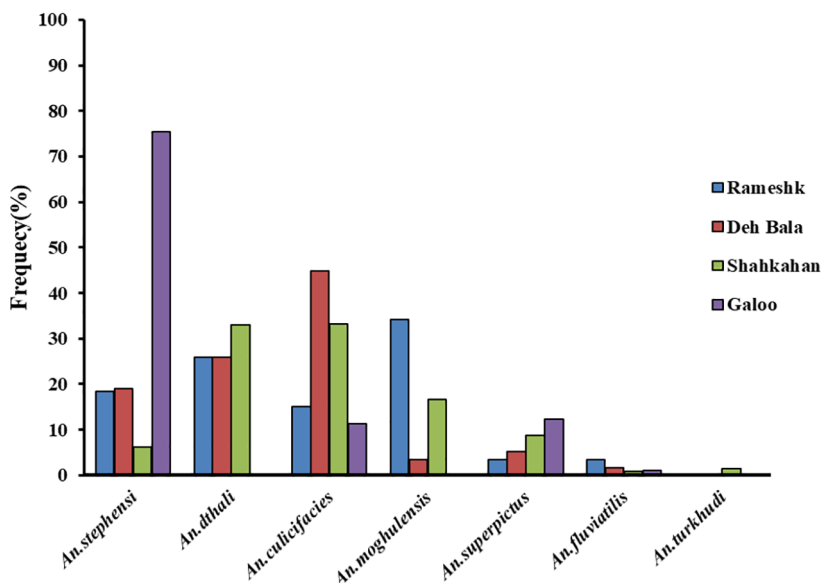


Figure 2: Frequency of species composition of *Anopheles* in Qaleh Ganj County, the southeast of Iran, 2018

Table 2: Physico-chemical characteristics of larval habitats in Qaleh Ganj County, the southeast of Iran, 2018

Parameters	Location			
	Galoo	Shahkahan	Deh-Bala	Rameshk
Kind of habitat	Water leakage	Fringe of the river	Fringe and bed of the river	Fringe of the river
Bedrock	Muddy	Stony and sandy	Stony	Sandy
Temperature of water	24	21-28	20-28	23-28
Temperature of air	29.7	28-36	28-38	31-40
Humidity	28	24-30	28-35	22-31
pH	7.1	5.5	5.6	6
Turbidity	Semi turbid	Clear	Clear	Clear
Light/Shadow	Shaded	Sunlight	Sunlight	Partial sunlight

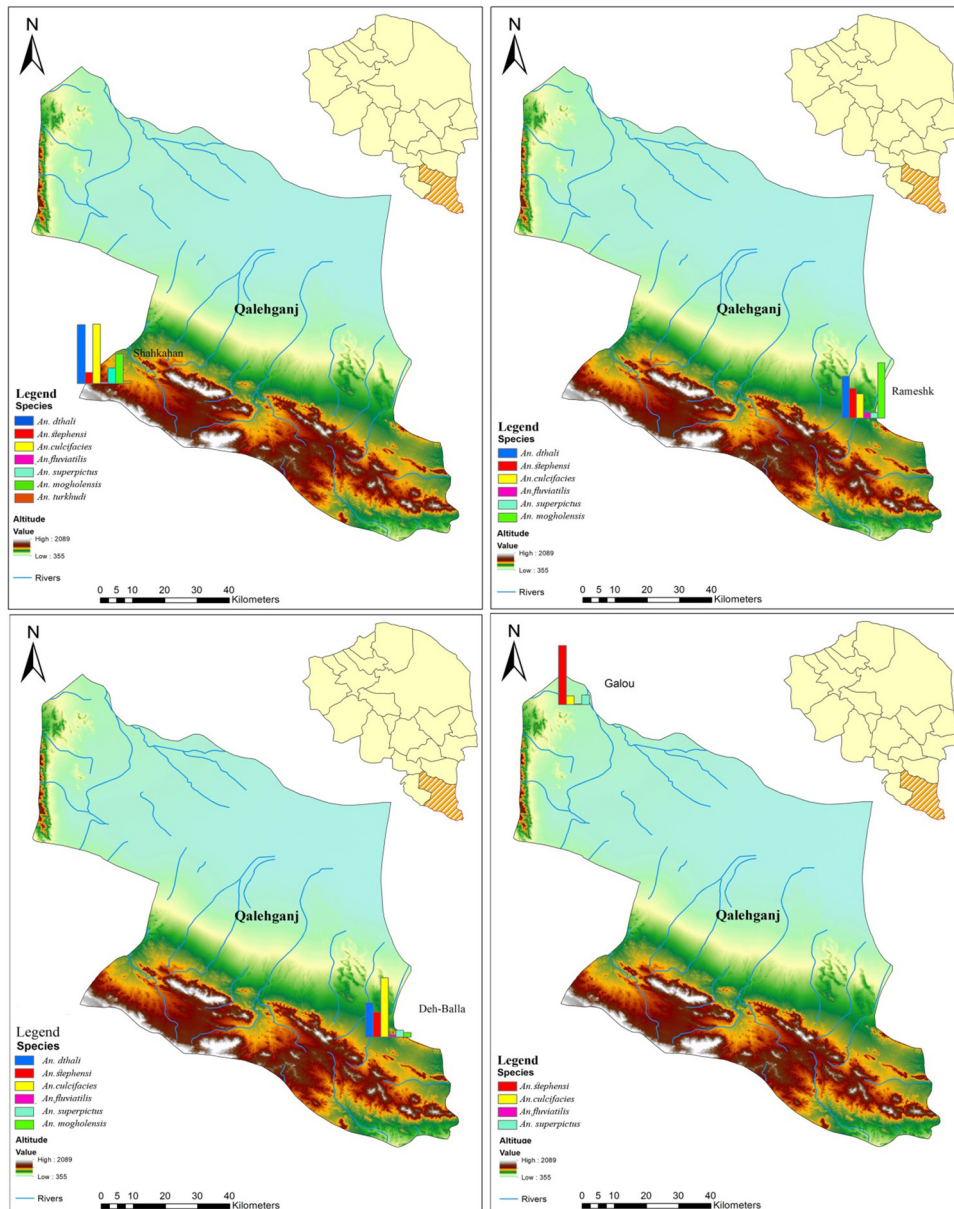


Figure 3: Spatial distribution of the collected species of *Anopheles* in Qaleh Ganj County, the southeast of Iran, 2018 (Designed by: Alireza Sanei-Dehkordi)

were investigated. Physico-chemical characteristics of *Anopheles* larval places from various habitats were determined (Table 2). Also, the typical Anophelinae larval habitats are illustrated in Figure 4.

Species Diversity

Some diversity indices such as Simpson's diversity index, Shannon-Weiner diversity index, and Pielou's evenness index were calculated (Table 3). Shahkahan area had the most richness among our sampling sites.

Discussion

In the present study, we reported the presence of seven species of *Anopheles* in our study area, including *An. dthali*, *An. culicifacies* s.l., *An. stephensi*, *An. moghulensis*, *An. superpictus* s.l., *An. fluviatilis* s.l., and

An. turkhudi. In addition to the species mentioned in our study, *An. apoci* has been reported in another study.¹² Our results about the species composition of *Anopheles* are in line with those of other studies conducted previously.¹³⁻¹⁹ Similar to another report, some of these species were reported earlier in Qaleh Ganj county.¹³

Anopheles dthali is distributed in the southern parts of the Zagros chain and coastal regions of the Persian Gulf in Iran.²⁰ It is also reported from Bushehr, Chaharmahal and Bakhtiari, Fars, Hamedan, Hormozgan, Ilam, Isfahan, Kerman, Kermanshah, Khuzistan, Kohgiluyeh and Boyer-Ahmad, Lorestan, Sistan and Baluchistan, and Yazd provinces.⁶ The temperature of the water in the breeding places ranges from 13 to 28 °C, with a pH of 6.9–8.⁸ *Anopheles dthali* is a secondary vector in some parts of Southern Iran, especially in elevated and mountainous regions

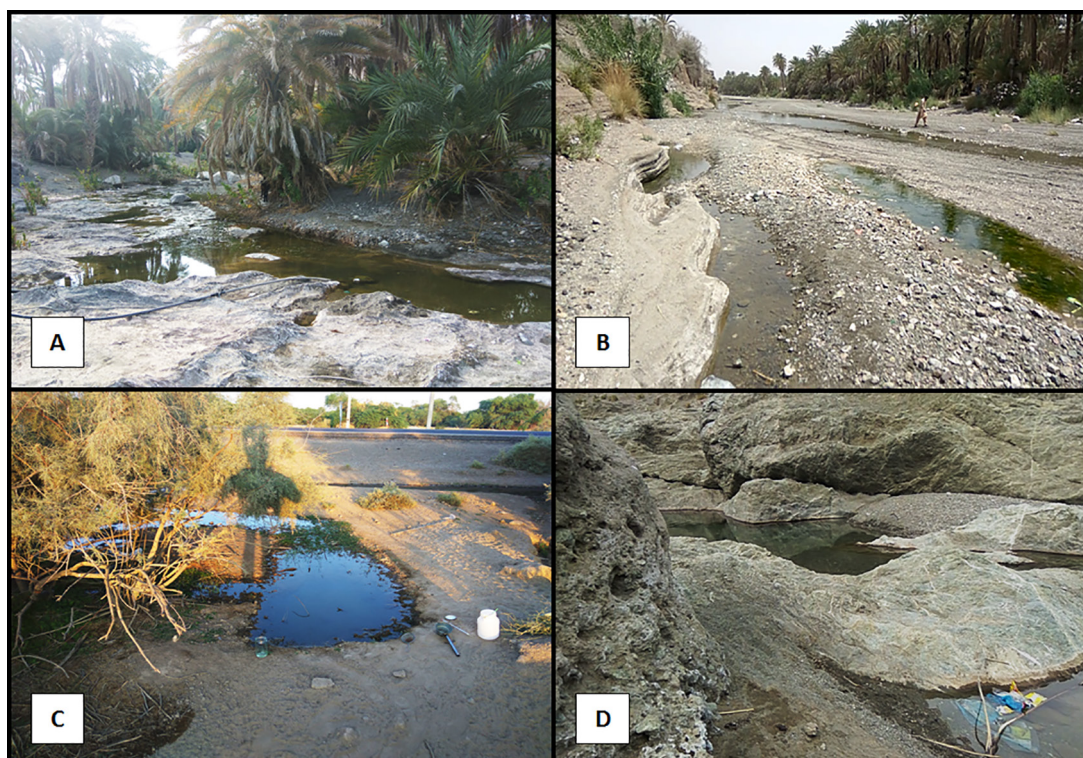


Figure 4: Typical Anophelinae larval habitats in Qaleh Ganj County, southeast of Iran, 2018. A. Shahkahan; B. Rameshk; C. Galoo; D. Deh bala; A and B: River edge, C: Water leakage, D: Riverbed (Photo by: Arsalan Amirkafi)

Table 3: Diversity, richness and evenness indices of each sampling area in Qaleh Ganj County, the southeast of Iran, 2018

	Number of larvae	Number of species	The Margalef's index	Simpson's diversity index	Shannon-Weiner's diversity index	Pielou's evenness index
Shahkahan	766	7	0.90	0.26	1.51	0.78
Rameshk	120	6	1.04	0.24	1.54	0.86
Deh-Bala	58	6	1.23	0.3	1.36	0.76
Galoo	98	4	0.65	0.59	0.76	0.55

of Hormozgan province.²⁰⁻²² Based on previous investigations, this species is often found in the fringe of the natural rivers with clear and full-sunlight waters and also distributed in permanent and stagnant water.^{8, 21} According to a recent study carried out near our study district, this species was recorded in Jiroft,¹⁰ Kahnuj, some parts of Qaleh Ganj Counties¹¹ as well as in Bashagard County located in the south of Qaleh Ganj. Based on previous studies, this species had a frequency of 17%⁸ and 18.02%.²¹ Our observation verified the presence of *An. dthali* in the area where this species has been reported previously.

Anopheles culicifacies s.l. is distributed in Sistan and Baluchistan, Kerman, Hormozgan, south of Khorasan, Bushehr, Khuzestan, and Fars provinces.^{23, 24} This species is the main malaria vector in the southeastern regions in Iran.^{25, 26} Rice fields in the Baluchistan district from April to September are the main larval breeding places of this species, and other places that look like irrigated palms and stream pools can also be the larval habitat of this species. During the winter months, from October to April, stream

pools serve as the predominant breeding sites of this species in most areas.²⁷⁻²⁹ *Anopheles culicifacies* s.l. is present mainly in Sistan and Baluchistan, Kerman and Hormozgan provinces.⁸ Several lines of evidence that were performed in adjacent counties of Qaleh ganj have shown the presence of this species in the fringe and bed of the rivers where it lays eggs in permanent and full sunlight habitats with clear waters look like *An. dthali*.^{8, 21} *Anopheles culicifacies* s.l. was recorded in Jiroft County,¹⁰ and another study found this species in Kahnuj and in some parts of Qaleh Ganj. It was also found in Bashagard County in the south of Qaleh Ganj.¹¹ A research conducted in Bashagard⁸ reported a frequency of 33.9%, and another research²¹ reported a frequency of 29.36% for this species. According to the information given above, the presence of *An. culicifacies* s.l. is logical in places where we found it.

Anopheles stephensi is the most common malaria vector in Southeastern Iran that is found with high abundance in many places, and it can oviposit wherever water is collected.²⁵⁻³⁰ This species breeds in many places of both urban and rural habitats in

all of its distribution regions. In rural areas, the breeding places are mostly pools, streambeds, the fringe of the rivers or streams, palm irrigation canals, waterfalls, and swamps with a slow water flow.³¹⁻³³ This species can be found in many localities of the south of the country (Hormozgan, Bushehr, Fars, Kerman, Ilam, Khuzistan, Kermanshah, Kohgiluyeh and Boyer-Ahmad, Lorestan, Sistan and Baluchistan provinces).^{6, 23} These characteristics show that it is an opportunist species that can reproduce and breed easily.²³ *Anopheles stephensi* was recorded in Jiroft.¹⁰ This species is also found in Kahnuj and some parts of Qaleh Ganj.¹¹ It was also found in Bashagard county in the south of Qaleh Ganj.¹⁹ An abundance of 13.3%⁸ and 5.01%²¹ was recorded for this species in two separate studies. According to the literature review about the biology and ecology of *An. stephensi*, the presence of this species is common in many larval habitats, even temporary ones.

Anopheles superpictus s.l. has the most dispersion in central plateaus, mountainous regions of the north of the country, and the hills of the south of the country.²³ This species was found in different altitudes from 50 to 2000 meters above the sea level.^{6, 34, 35} Larvae of *An. superpictus* s.l. can be found in many habitats or water bodies, especially sandy larval habitats of riverbeds with clear water, and also in prevalent habitats like fringe and bed of the rivers.^{6, 21} This species was recorded in all provinces in the country although there is no formal report from Qazvin province.⁶ This species was recorded in Jiroft County.¹⁰ Another study recorded the presence of this species in Kahnuj and some parts of Qaleh Ganj;¹¹ also, it was found in Bashagard County in the south of Qaleh Ganj.^{8, 21} According to this information, it seems very logical that we found *An. superpictus* s.l. in all our sampling habitats.

Anopheles fluviatilis s.l. is distributed in southern parts of the Zagros chain and the southern parts of Iran including Sistan and Baluchistan, Kerman, Khuzistan, Kermanshah, Fars, Kohgiluyeh and Boyer-Ahmad, Hormozgan, Ilam, and Bushehr provinces.^{6, 8, 23} It was also found in neighboring counties like Bashagard County in Hormozgan Province with very low abundance.⁸⁻¹⁰ This species breeds in different places like fresh, slow-flowing, or stagnant waters, the fringe of rivers, vast marshes, pits in the beds of stony or sandy rivers, and rainfalls.³⁶ In a research conducted in Bashagard county in the south of our study area,⁸ the frequency of this species was equal to 0.1%, but in another research,²¹ the presence of this species has not been reported. *An. fluviatilis* s.l. was found in Kahnuj¹⁰ and Jiroft.¹¹ In our research, this species was reported in low frequency.

Anopheles moghulensis is a non-vector species of malaria, so that there is little information about the biology and ecology of this species. According to this

little information, *An. moghulensis* seems to breed in the fringe of rivers,²¹ and it is also known as a species that prefers to live in mountainous areas.³⁷ *Anopheles moghulensis* has been even found up to an altitude of 1021 meters above the sea level.⁸ In the research conducted in the vicinity of our study area, *An. moghulensis* was recorded in Bashagard county with a frequency of 17.2%.⁸ In another study, this species was reported with a frequency of 25.2%.²¹ According to this information, and because Bashagard is adjacent to our sampling areas, the presence of this species is very normal.

Anopheles turkhudi is also a non-vector species and no comprehensive research has been done on bionomic aspects of this species. Anyway, according to a few studies, larvae of *An. turkhudi* are mostly found in permanent habitats where in most cases have vegetation, full sunlight with sandy beds, and sweet and clean water. The most natural breeding site for this species is the fringe of rivers.^{8, 38} It has previously been found in adjacent counties, including Jiroft and Bashagard Counties.^{8, 10, 21} In the research done in Bashagard county,⁸ *An. turkhudi* had a frequency of 7.2%; also, in another report²¹ this species had a frequency of 5.17%. *Anopheles turkhudi* has been found in our research in a small number in Shahkahan district which was close to Bashagard County.

According to the Shannon-wiener index, the highest level of species diversity was observed in the Rameshk district and also based on the Margalef's index, the highest level of species richness was observed in the Deh-Bala district. Based on the Pielou's index, Rameshk had the most evenness among our sampling regions.

It seems the high diversity of these mosquitoes in Rameshk district is due to the existence of permanent larval habitats and different species of domestic animals as blood sources for different species of mosquitoes, as well as the cool weather in this region caused by air masses coming from the east and higher altitudes.

According to Shannon-Weiner's and Simpson's indices, Galoo district had the least biodiversity among our sampling areas. While the number of samples collected in Galoo was sufficient, this phenomenon may be due to the temporary nature of the larval habitats in Galoo village. Dehbala and Rameshk villages had the most species richness among all the studied villages. The Pielou's evenness index was highest in Rameshk because the 120 collected specimens had more uniform distribution among the six species. Biodiversity indices were calculated in *Anopheles* species in our study area previously, but few studies have been conducted in other regions. One of the studies conducted in areas close to our study area⁸ calculated Shannon's index in Bashagard county in the vicinity of our study area, and their results

showed that this index ranged from 0.57 to 0.829, while it ranged from 0.76 to 1.54 in different parts of our study area. Also, another study³⁹ calculated Shannon's, Simpson's, Margalef's and evenness indices in Azerbaijan in the northwest of Iran. They calculated these indices for all their study area, but we calculated them for each region separately; moreover, it was about the Culicidae family, but our study was conducted on the Anophelinae subfamily.

Conclusion

In summary, based on the results of the current study, most of the *Anopheles* species recorded are potential vectors of malaria. Furthermore, these finding could be useful for implementing larval control programs and elimination of malaria in this endemic areas. In conclusion, given that some of the species in this region are species complexes, it is recommended that molecular studies should be conducted to find comprehensive information about the genetic diversity of these species.

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Conflicts of Interest: None declared.

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