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Spatio-temporal distribution of malaria vectors (Diptera: Culicidae) across different climatic zones of Iran

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ABSTRACT

Malaria is a main vector-borne public health problem in Iran. The last studies on Iranian mosquitoes show 31 *Anopheles* species including different sibling species and genotypes, eight of them are reported to play role in malaria transmission. The objective of this study is to provide a reference for malaria vectors of Iran and to map their spatial and temporal distribution in different climatic zones. Shape files of administrative boundaries and climates of Iran were provided by National Cartographic Center. Data on distribution and seasonal activity of malaria vectors were obtained from different sources and a databank in district level was created in Excel 2003, inserted to the shape files and analyzed by ArcGIS 9.2 to provide the maps. *Anopheles culicifacies* Giles *s.l.*, *Anopheles dthali* Patton, *Anopheles fluviatilis* James *s.l.*, *Anopheles maculipennis* Meigen *s.l.*, *Anopheles sacharov*i Favre, *Anopheles stephensi* Liston, and *Anopheles superpictus* Grassi have been introduced as primary and secondary malaria vectors and *Anopheles pulcherrimus* Theobald as a suspected vector in Iran. Temporal distribution of anopheline mosquitoes is restricted to April-December in northern Iran, however mosquitoes can be found during the year in southern region. Spatial distribution of malaria vectors is different based on species, thus six of them (except for *Anopheles maculipennis s.l.* and *Anopheles sacharovi*) are reported from endemic malarious area in southern and southeastern areas of Iran. The climate of this part is usually warm and humid, which makes it favorable for mosquito rearing and malaria transmission. Correlation between climate conditions and vector distribution can help to predict the potential range of activity for each species and preparedness for malaria epidemics.

1. Introduction

Malaria is one of the most important communicable diseases transmitted by anopheline mosquitoes (Diptera: Culicidae) to humans. This disease is endemic in 108 countries around the world. In 2008, there were an estimated 243 million cases of malaria causing 863 000 deaths, mostly of children under 5 years old[1]. It is one of the most infectious diseases in Iran with an average of about 15 000 annual cases in the last decade, while total recorded cases has dropped to about 3 000 in 2010 (Ministry of Health, annual reports). More than 80% of malaria cases in Iran are reported from three provinces of Sistan and Baluchistan, Hormozgan, and Kerman in southern and southeastern areas of the country[2]. There is another focus of the disease in northwestern Iran with an epidemic in recent years[3]. The most routes of malaria cases are immigration from Afghanistan and Pakistan to southern and southeastern areas of the country, as well as the Azerbaijan Republic in the northwest. The first study on malaria vectors was conducted about 90 years ago, when Latishev in 1921 identified *Anopheles maculipennis* Meigen *s.l.* (*An. maculipennis*) as the vector of malaria in northern areas of the country[2].

The genus *Anopheles* Meigen includes seven subgenera and at least 463 species and more than 50 unnamed members of species complexes[4]. About 70 species of them are malaria vectors in which about 40 are more important[5]. So far 31 species of the genus *Anopheles* representing two subgenera (*Anopheles* and *Cellia*), including different sibling species and genotypes, are recorded in the country, 16 out of them are in complexes or sibling species that some

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of them introduced as malaria vectors[6-9].

In southern areas of the country, *Anopheles culicifacies* (*An. culicifacies*) Giles *s.l.*, *Anopheles dthali* (*An. dthali*) Patton, *Anopheles fluviatilis* (*An. fluviatilis*) James *s.l.*, *Anopheles stephensi* (*An. stephensi*) Liston and *Anopheles superpictus* (*An. superpictus*) Grassi are known to be proven malaria vectors, while there is also report of sporozoite infection of *Anopheles pulcherrimus* (*An. pulcherrimus*) Theobald in southeastern Iran[2,10-12]. Also *An. maculipennis s.l.*, *Anopheles sacharovi* (*An. sacharovi*) Favre and *An. superpictus* are introduced as malaria vectors of western, northwestern and central plateau[2,11]. Recently, a study reported *Anopheles hyrcanus* (*An. hyrcanus*) Pallas (infected to *Plasmodium falciparum* in Guilan Province, northern Iran, by nested polymerase chain reaction assay[13], but more studies are needed to confirm this species as malaria vector in Iran. *Anopheles hyrcanus* Group needs to be studied extensively in the western Palaearctic Region including Iran[14].

Figure 1 shows the map of Iran. It has a variable climate affected by heat, humidity and clouds, rainfall, wind and dust, radiation and thunder, respectively. Based on these six factors, fifteen climatic zones including Southern boundary zone(1), Caspian hinterland area(2), Central Iran area(3), Azeri area(4), Khuzy area(5), Moghani area(6), Western Zagros region(7), Eastern Zagros region(8), Caspian littoral area(9), South hinterland area(10), Large Sistani area(11), Baluchi area(12), Makoui area(13), Small Sistani area(14), and High Zagros area(15) have been described[15](Figure 2). Also another (modified) classification demonstrates seven microclimates including: Arid (A), absolutely arid (AA), highly semi-arid (HAS), moderate semi-arid (MSA), slight semi-arid (SSA), semi-wet (SW) and wet (W) (Figure 2). Each climate provides favorite conditions for some *Anopheles* species. Average temperature in arid area is 11-14℃ and annual rainfall is 50-250 mm (Figure 2). Absolute Arid climate has average temperature and rainfall of 15-20 ℃ and 50-250 mm, respectively (Figure 2). Highly semi-arid climate contains areas with an average temperature of 7-10 ℃ and annual rainfall of 251-500 mm. In moderate semi-arid climate, average temperature and annual rainfall are 21-28 ℃ and 50-250 mm. Slight semi-arid climate covers areas with an average temperature of 7-10 ℃ and annual rainfall of 251-500 mm (Figure 2). Semi-wet climate is mainly located in average temperature of 7-10 ℃ and annual rainfall of 501-850 mm. Finally, the Wet climate has an average temperature of 0-10 ℃ and annual rainfall of 851-1 800 mm. Therefore in the northwest of Iran, winters are cold with heavy snowfall and subfreezing temperatures during December and January. Spring and fall are relatively mild, while summers are dry and hot. In the south, winters are mild and summers are very hot, having average daily temperatures in July exceeding 38 ℃. On the Khuzestan plain, summer heat is accompanied by high humidity. In general, Iran has an arid climate in which most of the relatively scant annual precipitation falls from October through April. In most of the country, yearly precipitation averages 250 mm or less. The major exceptions are the higher mountain valleys of the Zagros and the Caspian coastal plain, where precipitation averages at least 500 mm annually. In the western part of the Caspian, rainfall exceeds 1 000 mm annually and is distributed relatively evenly throughout the year. This contrasts with some basins of central plateau that receive 100 mm or less of precipitation annually (Figure 2).

Figure 1. Map of Iran.

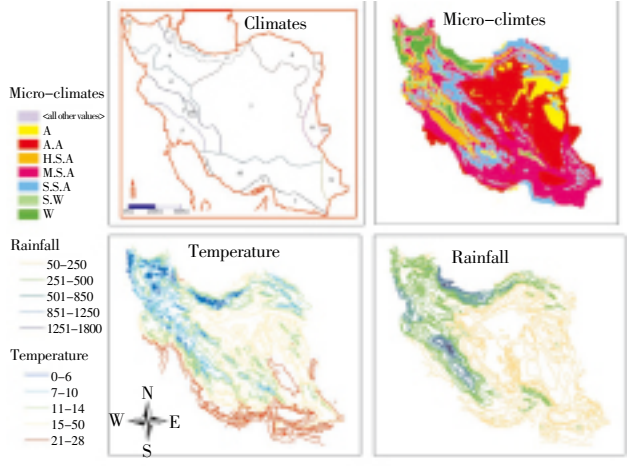

Figure 2. Climatic zones, microclimates, temperature and rainfall thematic maps of Iran.

The aim of the present study is to map the spatial distribution of main malaria vectors in different counties of Iran based on the reports of the previous studies, and to match it with different climatic variables and areas. Also in different climates, the spatial distribution of one *Anopheles* species may be affected by some factors such as preferred host accessibility, human activity, etc. The last shape files of administrative boundaries (including 31 provinces), thematic maps of rainfall and temperature, and also map of climates of Iran were obtained from the National Cartographic Center. A databank for distribution of malaria vectors was created in Excel software (Microsoft 2003) and then used for preparing the distribution maps in ArcGIS 9.2 software. These data were prepared and analyzed by ArcMap for spatial distribution mapping of the main malaria vectors at the county level in different climates and their temporal activity through the year.

2. Distribution of malaria vectors in different climatic zones of Iran

There are 31 *Anopheles* species including different sibling species and genotypes in Iran, eight of them are assumed to play role as malaria vectors. The present investigation emphasizes on the distribution of seven proven and one suspected vectors that are distinguishable morphologically, because most of obtained data were based on morphological characters. Therefore sibling species as the results of molecular studies are not included in our mapping.

In southern Iran from the west to the east, the weather is warm and humid and *Anopheles* species are active during the year (Figure 3). In other parts, the anopheline mosquitoes mainly start their activity during the spring, so that from June to October they can be found in all parts of their distribution range. After that from November temperature decreases in about 20 provinces, therefore in January minimum activity of Anophelinae can be observed (Figure 3).

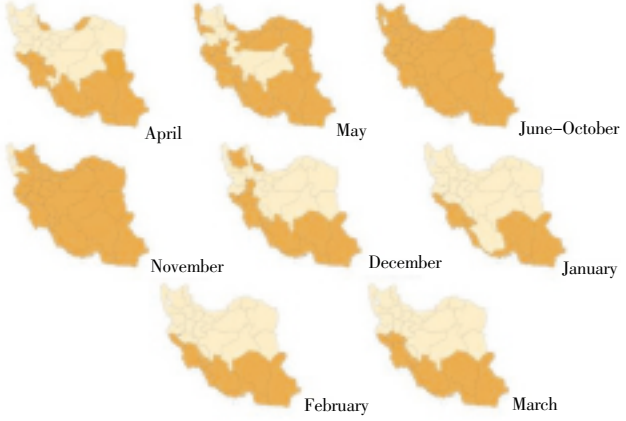

Figure 3. Temporal distribution of anopheline mosquitoes in Iran.

An. (*Cel.*) *culicifacies* Giles *s.l.* is the main malaria vector of Sistan and Baluchistan Province in southeastern Iran[10]. This complex consists of two species A and B (or probably a new species)[8,16,17] and is mainly distributed in southern and southeastern areas of Iran[6,18]. *An. culicifacies s.l.* occurs mainly in Sistan and Baluchistan, Kerman and Hormozgan Provinces. This species was implicated as a vector of malaria in Iran during an epidemic at Zabol (Sistan) in 1959[19]. It is the main malaria vector in southeastern corner of the country[11]. In Baluchistan the main peak of activity of the species occurs in April-May, whereas the secondary peak fluctuated from August to November. Study in southeastern Iran found considerable numbers of *An. culicifacies s.l.* resting outdoors in caves and pit shelters, with more than 40% of female freshly blood-fed specimens, suggesting high proportion of exophagy. The majority of endophagic females remained indoors for several days until becoming gravid. Human Blood Index (HBI) of this species in Baluchistan is reported from 1.18% to 20%. Sporozoite rate for this species is reported from 1% from Bandar Abbas in Hormozgan Province to 4.7% in Zabol, Sistan and Baluchistan Province[19]. Rice fields of Baluchistan area during April-September are the main larval breeding sites of *An. culicifacies s.l.*, along with irrigated palms and stream pools in other parts of its distribution area. During winter months, from October to April, stream pools serve as the predominant oviposition sites of this species in most areas[10,20]. The spatial distribution of this species is restricted to Bushehr, Hormozgan, Kerman, Sistan and Baluchistan, South Khorasan and Khuzistan Provinces. It mainly reported from moderate semi-arid and southern part of absolute arid climates (Figure 4)[6,8,10-12,18,21-27].

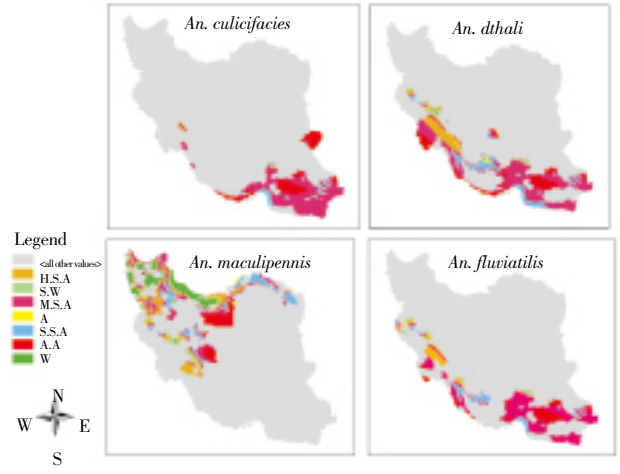

Figure 4. Distribution maps of *An. culicifacies s.l.*, *An. dthali*, *An. fluviatilis s.l.* and *An. maculipennis s.l.* in different climates of Iran.

An. (*Cel.*) *dthali* Patton is a secondary vector of malaria in southern Iran especially in mountainous areas of Hormozgan Province[28-30]. It has been found in southern parts of the Zagros chain, and coastal area of the Persian Gulf up to 1 410 m[29,30]. This species is widespread throughout the coastal and mountainous areas with high density in mountain areas. In these areas *An. dthali* is active throughout the year with two peaks, first in September-October and the other in April. This *Anopheles* is usually rests outdoors, although it is found in permanent human dwellings, tents, animal shelters, and food stores. Indoors, it has been collected from walls, and particularly from spider webs and hanging clothes. Outdoors, they are commonly found in natural shelters such as holes in river banks, rodent holes and old ruined Qanats. Precipitation tests on specimens from different parts showed up to 20.8% were positive for human blood. Anthropophilic index is varied between 1%-25% depend on locality. Sporozoite rates for *An. dthali* are reported from 0.67% to 2.08% in Iran. Larvae of this *Anopheles* were found in water with high salinity (2.7 parts per 1 000) in rural areas. *An. dthali* breeds in pebbly margins of rivers, springs, pits around springs with or without vegetation, pools in dried-up river beds, and palm irrigation canals in Hormozgan Province. In Bandar Abbas County, larvae were also found in mineral water. It is found in water with high salinity. The water temperature of breeding places ranging between 13℃ and 28℃, with a pH of 6.9-8.0. *An. dthali* is reported mainly from southern part of absolute arid, and parts of moderate semi-arid, slight semi-arid and high semi-arid climates (Figure 4). It is reported from Bushehr, Chaharmahal and Bakhtiari, Fars, Hamedan, Hormozgan, Ilam, Isfahan, Kerman, Kermanshah, Khuzistan, Kohgiluye and Buyerahmad, Lorestan, Sistan and Baluchistan, and Yazd Provinces (Figure 4)[6,8,11,12,18,21-27,29-33].

An. (*Cel.*) *fluviatilis* James *s.l.* is introduced as a secondary vector in southern and southeastern areas of the country[11]. In *An. fluviatilis* complex the species T, U and V are reported based on rDNA ITS2 and D3 sequence analysis in Iran[8,9], though Naddaf *et al*[34] were in doubt about the

occurrence of the species V. This *Anopheles* is distributed on the southern slopes of the Zagros chain, from southwest of Kermanshah Province to Sistan and Baluchistan Province in the southeastern part of Iran, and found at altitude from 50 meter to 1 100 meter. Its seasonal activity and maximum density has two periods, from April to June and from late September to December on plains and from the end of spring up to the beginning of autumn in foothill area. This species is reported both endophilic and exophilic, although in some areas it shows a marked exophilic tendency. Due to the outdoor sleeping habits of the local population in this area during warm months of spring and summer, this species is considered as exophagic. Human blood index of this species is reported from 2.68% to 28.6% in different ecological zones. Sporozoite rate for this *Anopheles* is recorded 1.4%-11%. *An. fluviatilis* breeds in fresh, slow flowing or stagnant waters, vast marshes, river banks, pits in the beds of stony or sandy rivers and rainfall pits[35,36]. This species is reported from Bushehr, Fars, Hormozgan, Ilam, Kerman, Kermanshah, Khuzistan, Kohgiluye and Buyerahmad, Lorestan and Sistan and Baluchistan Provinces, where climates are moderate, slight or high semi-arid (Figure 4)[6,8,11,12,18,21,23,24,31,35,37].

An. (*Ano.*) *maculipennis* complex comprises 7 species in Iran: *Anopheles atroparvus* van Thiel, *Anopheles labranchiae* Falleroni, *An. maculipennis, Anopheles melanoon* Hachett, *Anopheles messeae* Falleroni, *Anopheles persiensis* Linton, Sedaghat and Harbach, and *An. sacharovi*[6,8]. In the Maculipennis Group only *An. sacharovi* can be distinguished morphologically from others[38-40], thus most of published data introduced *An. maculipennis s.l.* and *An. sacharovi* as two species that are included in malaria transmission in northern and northwestern areas of the country[2,3,11] based on a morphological key[39]. *An. maculipennis* complex has been identified as the major vector of malaria in the Caspian Sea littoral. Edrissian *et al*[41] and Vatandoost *et al*[42] reported HBI of 4.9%-5% for *An. maculipennis s.l.* Sporozoite rate for this species is reported 0.33%. *An. maculipennis s.l.* has endophilic and endophagus behavior. Larval habitats are including clear samples along the edges of slowing rear-ends of water supply channel and roadside ditches, as well as rice fields. Its seasonal activity is mainly between late May to October with a peak in August[43]. There is no report of this species from Bushehr, Hormozgan, Kerman, Ilam, Sistan and Baluchistan, South Khorasan, Qom and Yazd Provinces (Figure 4). This *Anopheles* is mainly distributed in wet, slight semi-arid, moderate semi-arid and high semi-arid climates, although there are reports of existence in absolute arid climate (Figure 4)[3,6,8,18,21,32,33,37,40,42,44-59].

An. (*Cel.*) *pulcherrimus* Theobald is reported serologically positive to *Plasmodium falciparum* and *Plasmodium vivax* in Sistan and Baluchistan Province and introduced as a suspected vector in that area[10,12]. Slow moving streams, ditches, rice fields, pools, marshes and other types of waters with or without vegetation have been reported as the breeding sites for *An. pulcherrimus* larvae. However, rice fields, weedy irrigation channels, marshes and any kind of clean stagnant water, overgrown with vegetation, but not too heavily shaded, have been reported as the most favorable sites[60]. The species had been active throughout the year with one peak of activity in August-September in Sistan and Baluchistan Province[10]. Human blood index of this species is reported 1.12%-9.9%, while its sporozoite rate was 3.5% in Qasre-qand, Sistan and Baluchistan Province[22,41]. This species is mostly exophilic, but a part of the population also uses indoor resting sites[60]. *An. pulcherrimus* occurs in moderate and highly semi-arid climates[15] (Figure 5) in Chaharmahal and Bakhtiari, Fars, Golestan, Hormozgan, Ilam, Kermanshah, Khuzistan, Kohgiluye and Buyerahmad, Mazandaran, North Khorasan, and Sistan and Baluchistan Provinces[8,11,12,18,22,23,31,37,58,60].

An. (*Ano.*) *sacharovi* Favre is a major vector of malaria in northwestern Iran and is distributed in foothills of Zagros Mountains from northwestern, western and southwestern areas of Iran, as well as northern belt. It is a member of *An. maculipennis* complex and only this species can be distinguished morphologically from other members of this complex in adult and larval stages[38,39]. This species is mainly endophilic and endophagus, although the relative exophilicity is also reported. Based on the survey of Yaghoobi-Ershadi *et al*[3] this species showed 38.5% blood fed on human alone and 23% on both human and dog in human dwellings, but the specimens that had fed on the animal shelters were negative for human blood. The anthropophilic index of *An. sacharovi* has been reported between 4.2% and 30.6% from Iran in 1969 but the sources of their blood meal smears were not stated[43]. Human blood index values of 26.5% from human dwellings and 9.4% from animal shelters were observed from East Azerbaijan and Khuzistan Provinces of Iran during 1982-1984[41]. Soporozoite rate for this *Anopheles* is reported 1%-2%[43]. Larval habitats include clear samples along the edges of the slowing rear-ends of water supply channel and roadside ditches, as well as rice fields. Seasonal activity starts from April and extends to October-November, with a peak in July-August in southern Iran, while in the northwest of the country it is active from late June to late November with one peak in late August[3]. This species is reported mainly from wet, moderate semi-arid and high semi-arid climates in Ardebil, East Azerbaijan, Fars, Guilan, Golestan, Kermanshah, Khuzistan, Kohgiluye and Boyerahmad, Kurdistan, Lorestan, Mazandaran, Qazvin, Tehran, and West Azerbaijan Provinces (Figure 5)[3,6,8,11,21,37,38,42-46,50,55,57,61,62].

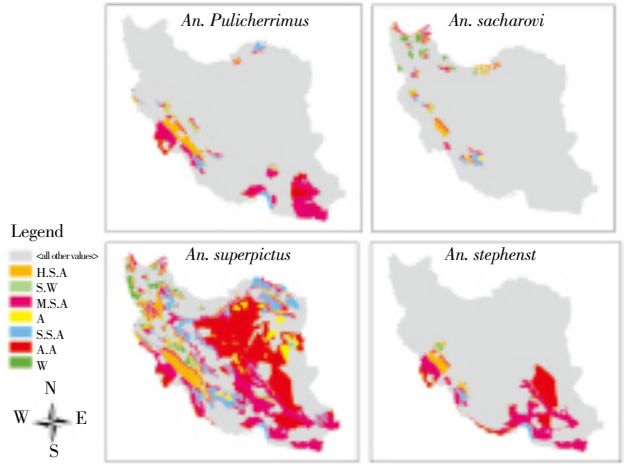

Figure 5. Distribution of *An. pulcherrimus, An. sacharovi, An. stephensi* and *An. superpictus* in different climates of Iran.

An. (*Cel.*) *stephensi* Liston is the main malaria vector in southern coastal areas of Iran[2,11,63]. The species includes three egg phenotypes: *mysorensis*, typical and intermediate, all exist in Iran[7]. These egg phenotypes are natural

variations and systematically considered infrasubspecific forms of the species[8,24]. The previous investigations have shown it to be the most prevalent anopheline species in the malarious area of southern Iran up to altitude of 900 m[64], although recent studies extended its distribution up to 2 000 m in Lorestan Province[32]. *An. stephensi* is active during the year in southern coastal area, with two peaks in April-May and September-October. It can be found from April to November in cold mountainous area with one peak of activity in July. This species is endophagous and endophilic. There are different reports of anthropophily for this species and HBI is recorded 0.5%-3.6% in different counties. Sporozoite rate of samples from the southern areas of Iran was reported from 0.2% to 1.8%. *An. stephensi* breeds in a wide range of both urban and rural habitats throughout its distribution region. In urban areas this species breeds in all sorts of water bodies, such as wells, cisterns, fountains, ornamental ponds, and in water used for building construction. Larvae can be collected from ponds, pools, stream margins, catch basins, and seepage canals. It is found in water with high salinity, sometimes reaching or even exceeding that of seawater. In rural areas, the breeding places are pools, streambeds, palm irrigation canals, at the margin of streams and rivers, seepages, and marshy areas with a gentle water flow[64,65]. This species is distributed mainly in moderate semi-arid and absolute arid climates, although high semi-arid and slight semi-arid climates are also included. This species is reported from Bushehr, Fars, Hormozgan, Ilam, Kerman, Kermanshah, Khuzistan, Kohgiluye and Buyerahmad, Lorestan, Sistan and Baluchistan Provinces (Figure 5)[6-8,11,12,18,21-24,31,32,37,43,64,66].

An. (*Cel.*) *superpictus* Grassi is considered as a major malaria vector in central plateau, and the secondary one in the southern areas of the country[2,11]. Oshaghi *et al*[67] reported two distinct morphological forms (A and B) of this species from Iran, while molecular analysis of mitochondrial DNA COI-COII region separated this *Anopheles* into at least three genotypes X, Y and Z[68]. *An. superpictus* has a widespread distribution in Iran across the all climatic zones, from 50 to 2000 meter. It is reported from absolutely arid to wet climates (Figure 5). It is active during April to November is the most parts of the country with one peak in August-September. It is exophagic with both exophilic and endophilic behavior. Human blood index and sporozoite rates for this *Anopheles* are 11.4% and 0.65%-4.7%, respectively. Larvae of *An. superpictus* can be found in different water bodies with ground origin, especially sandy larval habitats of riverbeds with clear water. The species is recorded in all provinces of the country, however there is no formal report of it from Qazvin Province[3,6,8,11,12,18,21,23,31-33,37,41,42,44,46,47,49,50,53-55,57,58,67-70].

According to the present investigation, *An. superpictus* is found in almost all Iranian climates and that may explain why the species is collected from all areas of the country (Figure 5). This makes the species an important malaria vector in Iran especially in central plateau. *An. culicifacies s.l.*, *An. fluviatilis s.l.*, *An. pulcherrimus* and *An. stephensi* are found in 5-6 (out of 15) climates based on Masoodian's[15] classification or 2-4 climatic zones based on modified classification. These species are not collected from central and northern Iran except for *An. pulcherrimus* (Figure 4,5). There is an exceptional distributional focus of *An. pulcherrimus* in northeastern Iran (Figure 5). This is interesting in view of its occurrence in Middle Asia[58]. Also, *An. dthali* is not recorded in central and northern Iran, though it is found in 9 (out of 15) of Masoodian's climatic zones[15] and 4 (out of 7) modified classification (Figure 4). The general distributions of *An. dthali, An. fluviatilis s.l.*, and *An. stephensi* seem to be similar in southern Iran (Figure 4,5) where they are the main and secondary vectors of malaria. However the distribution of *An. culicifacies s.l.* mostly occurs in the southeastern wards of the country (Figure 4) where it is known the main vector. *An. maculipennis* Group occurs in different climatic zones, 9 (out of 15) of Masoodian's climatic zones[15] and 5 (out of 7) modified classification for *An. maculipennis s.l.* and 8 (out of 15) and 3 (out of 7) for *An. sacharovi* respectively, and the group has a wide distribution in central, northern, western, and even southwestern Iran, however it does not occur in southeastern Iran and the southern part of the country (Figure 4,5). This is the reason *An. maculipennis s.l.* and *An. sacharovi* do not play role in malaria transmission in the main malaria foci in southern and southeastern Iran, however they are the main vectors in northern foci. Exact distribution of all members of the group is not clear because of problematic morphological identification of them.

Although some species are found in similar climatic zones, they show different distribution or vice versa. The reason may be explained that the climatic zones are described here mostly based on temperature and rainfall and other environmental and biological features which influence distribution and abundance of mosquitoes such as altitude, water salinity influenced by soil texture and structure, larval habitat characteristics, plants, preferred hosts and so on need to be investigated more to answer the questions. Also, what we find out about the distribution of the species today has been critically influenced by human activities and climate change especially in recent decades.

Using climatic data and ecological information about favorite conditions for breeding of different malaria vectors, GIS may predict the probable distribution areas of each species. This action needs a database consisting of ecological parameters which are suitable for breeding and survival of anopheline vectors of malaria. Although study on the fauna, biology, ecology, susceptibility status to insecticides and molecular taxonomy of *Anopheles* mosquitoes has an old history and information about anopheline mosquitoes in Iran has been collected during about 90 years, GIS-based studies are rare. This method can help entomologists to work more careful and will facilitate decision making for malaria control programs. Global warming can significantly impact on the distribution and severity of malaria on a global level, especially as a mosquito-borne disease which is highly sensitive to changes in climate. So developing models for distribution of *Anopheles* species based on environmental conditions and ecology of vectors will help us to have up-to-date maps for each species.

More studies are needed to create a well described database on biology, ecology, systematics and susceptibility status of malaria vectors at larval and adult stages in Iran. We recommend collecting such information from other vector-borne disease in the country for preparedness and early warning for probable epidemics especially in the natural disasters for implementation of any vector control measures.

Conflict of interest statement

We declare that we have no conflict of interest.

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